Models and Seroepidemiology of

Co-existing Virus Strains.

by

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This thesis is dedicated to the memory of

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Abstract.

This thesis investigates the effect of interactions between the immune system and co-existing viruses on the dynamics of viral infections, such as influenza. Specifically, records from the United Kingdom and the United States of America are examined, which indicate that cross-immunity between two co-existing viruses may lead to a predominance of one strain over another during annual epidemics. Parameters for the 'force of infection', 'cross-immunity coefficient', infectious period and age-dependant contact rate for two co-existing strains of influenza are estimated from both case notification and serological data.

Time series analysis techniques are used to examine the evidence of oscillatory secular trends in the incidence of influenza virus strains. Only seasonal effects are found to contribute to the long-term fluctuation of the virus.

Analysis of data collected in a large survey (sample size 3000) of antibody directed against the influenza virus in North Yorkshire, finely stratified by age, reveal age-dependant patterns in the rate of virus transmission. The rate of infection changes from high in older children and young adults to low in the middle age groups, and then high again in the elderly age classes. Mean antibody levels are considered with respect to age, and the variability of these levels are also discussed.

Horizontal cross-sectional data are studied for a number of years, giving an understanding of the build up of immunity for both an 'endemic' strain, A/Eng/333/80, and two 'epidemic' strains, A/HK/68 and A/Bel/1/81. The changes in transmission rates with respect to time are discussed.

Based on the direct transmission properties of the influenza virus a mathematical model is proposed to describe the role of cross-immunity in the interractions of co-existing viral strains of humans. The parameters are estimated from serological and reported data, and it is shown that, by using these parameters in the numerical analysis of a series of eight coupled differential equations, it is possible to generate epidemic patterns similar to those observed.

Expressions for the Basic Rate of Reproduction and the Average Age at Infection are derived from the model, and the inherent stability of these expressions under different parameter conditions are explored.

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Chapter One: General Introduction

1.1. Introduction.

This chapter presents an introduction to the biology and epidemiology of the influenza virus and to the general concepts that underpin the research described in this thesis. It provides a brief review of the subject, and gives a background to the research that has been conducted, in terms of data collection and analysis, and to the interpretation of the epidemiological results described in the thesis.

The aims of this study are to investigate the possible mechanisms responsible for the persistence of co-circulating virus types between pandemics, as well as their role in fostering the recurrence of epidemics. Specifically, age-structured case notifications and serological surveys within populations are used to determine whether interactions between the immune system and co-existing viruses play a part in the transmission dynamics of the influenza virus.

The influenza virus causes disease in over 30% of the population of the United Kingdom per year, with 25% of all respiratory illnesses due to infection with the virus. It is estimated that over 10% of the population seek medical help each year due to influenza induced disease, 0.12% are hospitalised and approximately 0.005% to 0.01% of the population of Britain dies from influenza each year (Couch et al. (1986)).

In both the UK and the USA influenza epidemics occur in the winter of every year, with a peak of respiratory disease coinciding with a peak in the incidence of influenza viruses (Chakraverty et al. (1986)). In most cases two distinct strains can be observed in each epidemic, with one arriving slightly earlier, peaking higher, and also finishing before the second virus, which gave rise to the 'Herald Wave' theory (Glezen (1982), Glezen, Couch and Six (1982), and Glezen et al. (1980)).

The phrase 'Herald Wave' is used to describe a wave of influenza virus infections which occurs during the second half of the epidemic period and which it was thought heralded the epidemic virus for the following years (Glezen (1982)). In the publication by Glezen (1982)

the data was presented such that this wave appeared to occur for three seasons in succession. However, it represented only two 'herald' waves denoting the emergence of the new strain for the successive season, and not three seasons as was suggested. After these two herald waves, no others were recorded. The study by Glezen, however, presents some data for the United States which agrees with that for the United Kingdom (Chakraverty et al (1986)), showing that there are consistently two strains of influenza causing infection in the same season. It is possible that the consistent patterns observed are due to an interaction between the two co-existing strains, where cross-immunity prevents the second strain from causing a significant epidemic in the presence of the first strain. This is not necessarily due to immunity conferred by one strain on the other in the same season, but could be due to cross-immunity from a previous, similar strain, preventing the latter strain from causing an equivalent number of cases. This effect could also be due to differing rates, or forces, of infection (due to differing transmissibilities or infectiousness of the virus strains). Further data of this kind from the United Kingdom (presented in detail in chapter 3) shows that two strains of the same subtype rarely co-exist in the same season.

It is thus probable that the two concepts, co-existence and the emergence of a new virus strain in most seasons, are related. Obviously some strains of virus will be more successful than others and possibly survive for more than one season, which will cause several strains to co-exist at one time. However, strains which are antigenically similar to previous strains will have a considerably reduced pool of individuals who are susceptible to infection, and will therefore not cause as many cases as those strains that are unique in their antigenic presentation.

Many aspects of the influenza virus have been examined in much detail. These include the molecular structure of the virus (Both et al. (1983) and Webster et al. (1982) for example) which changes through time as a result of exposure to and selection by the human immune system (Oxford et al. (1980) and Couch and Kasel (1983) for example). Herd immunity, in defined communities, causes the epidemic patterns observed in these communities, and these patterns have been the subject of much attention by epidemiologists (e.g. Stuart-Harris et al. (1985), Hope-Simpson (1981), Glezen (1982) and others). There have also been attempts to determine the nature of the rates of transmission that determine observed patterns by utilis-

ing mathematical models (Elveback et al. (1964)). However, to date research has not fully elucidated the processes behind the dynamics of the influenza virus. Many questions about the epidemiology of the virus, therefore, remain unanswered. These include the following topics.

Where do viruses 'hide' and what causes them to emerge? What is the origin of the subtypes, and why do they always appear to emerge in the Asian continent ? Why are shifts in the antigenic presentation restricted to type A influenza? Does antigenic drift continue indefinitely? Why does no natural antigenic drift occur in other myxoviruses, especially considering that it can be induced in measles, using monoclonal antibodies, at about the same frequency as influenza (Webster et al. (1982))?

One of the greatest puzzles related to the influenza virus is the differences observed between the dynamics of the virus in human populations and the dynamics of other myxoviruses (e.g. mumps, measles and rubella) which have the same basic structure (see section 1.2.1). Influenza is the only myxovirus which, due to continuously changing antigen presentation, is in effect continually being introduced into a large pool of susceptible individuals. This causes a large epidemic and, therefore, a considerable proportion of the population becomes immune to each specific strain in a short interval of time after the introduction of that strain. This appears to prevent a given subtype of the virus from persisting in any given host population over long periods of time. In other words, the apparently high reproductive rate of the virus results in a 'boom and bust' pattern where, following arrival, a given strain quickly exhausts the supply of susceptible hosts.

In this thesis the influenza virus is used as an example of an infectious agent with coexisting strains. It does not attempt to explain all the multi-faceted concepts involved in the transmission of influenza viruses. It is hoped, however, that some of the ideas generated by this research may lead to a better understanding of the epidemiology of this fascinating infectious agent.

1.2. Structure of the virus.

1.2.1. Nomenclature.

Influenza occurs in three distinct types; A, B and C. Of these three types influenza A is the only one which is further divided into subtypes, determined by the structure of the (antigenic) surface protein (as discussed in section 1.2.2). The subtypes can also be separated due to small differences in the surface protein which allows the differentiation into different strains of the influenza virus. Of the three distinct types, only A and B are of epidemiological significance. Due to the variability of the surface protein the virus possesses a considerable potential for genetic variability, which gives rise to the altered antigenic determinants causing the strains (see section 1.2.3). It is these subtypes and strains which cause the recurrence of pandemics and epidemics respectively.

The nomenclature of influenza viruses has been under constant revision since 1933 (when the virus was first isolated). In the system of nomenclature for influenza viruses recommended by the World Health Organisation (WHO) in 1971 (WHO (1980)) the viruses were classified into types, (A, B and C) on the basis of the antigenic character of the nucleoprotein (NP). Type A viruses were further divided into subtypes based on the antigenic character of their haemagglutinin (HA) and neuraminidase (NA) antigens. The haemagglutinin antigen subtypes of human influenza A viruses were further reviewed by the WHO in 1979 and 1980 which led to the current system of nomenclature, which ensures that the strain designation for influenza viruses contains the following information:

1) The type of virus based on the antigenic specificity of the NP antigen (type A, B or C).

2) The host of origin for the strains (if not isolated from human sources).

3) The geographical origin.

4) The strain number.

5) The year of origin.

6) (For influenza A only) an index describing the antigenic character of the haemagglutinin (H1, H2 or H3).

7) (For influenza A only) an index describing the antigenic character of the neuraminidase (N1 or N2).

It is implicit in this system that a given haemagglutinin or neuraminidase subtype designation will encompass strains exhibiting some antigenic variation within the subtype (known as 'antigenic drift' (Webster et al. (1982)). The exact antigenic character of an influenza strain may be defined by indicating similarities between the strain in question and a designated reference strain. The 1980 nomenclature system was not designed to provide information on the host range or virulence of influenza viruses.

1.2.2. Virus structure.

Influenza is an enclosed virus with five internal non-glycosylated proteins, and two external glycosylated proteins. The five internal proteins consist of the matrix protein (M), the nucleoprotein (NP), and three polymerases. The external proteins are haemagglutinin (HA) and neuraminidase (NA).

Immunity is induced in response to an immunogenic stimulation by the two glycosylated external proteins, especially haemagglutinin. This view has been reached from clinical investigations with live and attenuated virus vaccines and with 'wild' virus challenges. Field observations and animal models also support this theory (Greenwood et al. (1936)). New subtypes occur due to the sudden 'shifts' in the molecular structure of the HA, which usually cause pandemics, with later strains being developed after more subtle 'drift' in the HA, leading to localised epidemics (see section 1.2.3. for details).

The haemagglutinin molecule possesses four non-overlapping antigenic regions consisting of clusters of epitopes, whereas neuraminidase has three overlapping regions. Primary sequences of haemagglutinin from H3N2 viruses collected since 1968 show that each new strain has at least one amino-acid substitution in each of the four antigenic areas (Couch and Kasel (1983)). The viruses are classed as A, B, or C due to their matrix and nucleoprotein type-specificity. There are thirteen distinct haemagglutinin sub-types and nine distinct neuraminidase subtypes in the influenza virus.

The highly pleomorphic particles of influenza virus are enclosed in a lipid membrane derived from the plasma membrane of the host cell. Haemagglutinin and neuraminidase antigens are attached by short sequences of hydrophobic amino-acids at one end of the mole-

cules (Webster et al. (1982)). Some carbohydrate side chains show host-cell related antigenic activity.

The matrix protein is structural and contains eight single-stranded ribonucleic acid (RNA) molecules which are complementary to the messenger RNA, which is to say that they are 'negative' RNA, and are associated with a nucleocapsid protein and three large proteins, P1, P2 and P3, which are responsible for RNA replication and transcription. Three virus-encoded non-structural proteins are also found in the host cells, but their function is still unknown (Webster et al. (1982)).

1.2.3 Variability in Virus Structure.

The changes in antigenicity of viruses appears to be driven by the need to overcome immunity, either natural or induced by vaccination, and escape from immunity has been achieved by the haemagglutinin alterations (Both et al. (1983)). The HA undergoes frequent and progressive antigenic drift as a result of selection, under immunological pressure, of viruses possessing alteration in the amino acid sequences at specific sites in the molecule (Schild et al. (1983)). If immunological changes are sufficiently large, they enable a descendant of the influenza virus strain to reinfect hosts that are immune to the progenitor influenza strain and hence to invade communities that recently suffered an epidemic of the progenitor strain (Pease (1985)).

'Drift' and 'shift' are used to distinguish two alternative mechanisms of evolution in the influenza virus. Drift occurs by point mutations and possibly by short deletions and insertions (Palese and Young (1982)) which leads to the accumulation of amino acid sequence changes that alter the antigenic sites in such a way that they are no longer recognised by the host's immune system, while shift occurs when a completely novel influenza serotype is introduced into the influenza population which infects humans. This novel influenza strain may arise from the influenza serotypes previously infecting swine, equine or waterfowl populations (Palese and Young (1982) and Webster et al. (1982)). Drift occurs continuously, causes gradual changes in the influenza antigens, and is responsible for most of the observed year to year variation in the influenza antigens. Shift occurs irregularly (as in 1957, 1968 and 1977) and causes abrupt and large changes in the influenza antigens. Drift as used

by molecular immunologists is not equivalent to the concept of genetic drift in population genetics. Within the context of influenza biology, drift is used to describe any gradual change in influenza antigens (Webster et al. (1982)). These gradual changes may be caused either randomly or by a type of frequency-dependent selection which occurs because variant influenza viruses have a selective advantage when they first arise (and are thus rare), since fewer hosts will have derived an immunity to them than to the more abundant prevailing strains.

Large proportions of the population becoming immune causes the variant viruses to have a selective advantage, and hence more rapid rate of amino acid substitution, than they would have in a largely susceptible population (Pease (1985)).

Thus there is a significant selection pressure for new variants to evolve, either by major changes to the surface antigens (shift) or by less sudden, smaller changes to the surface proteins (drift). It is interesting to note that the same pressure exists with respect to other myxoviruses, which are endemic in the host population, yet such considerable variation is only found in the influenza virus.

1.3. The History of Influenza.

In 1933 di Camagliano referred to the astrologers' view that the conjunction of planets 'influences' the epidemics of fever, coughs and colds in the city of Florence, while Thomas Willis (1852) noted that the 'outbreaks occurred as if sent by some blast of the stars' (Stuart-Harris, Schild and Oxford (1985)).

It is known that a disease with symptoms which closely resemble the symptoms of influenza has been observed from the twelfth century onwards and it is possible that it was first discovered in 1510 (Vaughan (1921), Beveridge (1977)). However, the first case description officially recorded as 'influenza' was in 1557 and the influenza virus was not discovered to be the etiological agent of this disease until 1933 (Smith, Andrewes and Laidlaw (1933) in England, and Francis and Magin (1936) in the USA). Retrospectively a serological relationship has been determined between prototype human and swine viruses, which suggests that the human population first experienced a major epidemic due to the transfer

Table 1.1. The incidence of influenza A subtypes (based on the surface protein composition) from 1933 up to 1989 (see text for data sources).

Years Recorded	Subtype.
1933-1945:	H0N1 (Later classified as H1N1)
1946-1956:	H1N1
1957-1967:	H2N2
1968-1976:	H3N2
1977-1989:	H3N2 + H1N1 (mostly in youths).

between these two species in 1918 (Webster et al. (1982)). Since then only four major subtypes have caused all the epidemics of human influenza, each in isolation until 1977. Table 1.1. shows the occurrence of subtypes from 1933 to 1989.

Initial evidence of the occurrence of a new subtype (caused by a sudden shift in the molecular structure of the major antigenic determinant) is usually in the form of a pandemic, with later, more localised, epidemics being caused by genetic drift. These epidemics and pandemics always occur seasonally and almost invariably no viruses are evident in the summer months (Hope-Simpson and Golubev (1987)). During this century there have been four distinct pandemics: the first in 1918, the H1N1 subtype; the second in 1957, due to the H2N2 subtype ("Asian 'flu'"); another in 1968 after the appearance of the H3N2 subtype ("Hong-Kong 'flu'") (Webster et al. (1982)); and the last in 1977 caused by the subtype H1N1 ("Russian 'flu'") (Both et al. (1983)).

1.4. Epidemiology.

1.4.1. Basic Observations.

There are three main methods by which a new influenza epidemic may occur. A previously 'hidden' virus may begin to recirculate; a new strain may appear which is derived from an animal or avian strain; or an avian or animal strain may become infectious to humans (Webster et al. (1982)). Influenza has been monitored by the World Health Organisa-

tion (WHO) since 1947. This surveillance stems from frequent episodes of disease throughout the world and the association of epidemics and pandemics with genetic drift and shift respectively, plus the high mortality which is sometimes associated with pandemics. The study of influenza isolated in northern and southern hemispheres in respective winter seasons reveals that alterations can be detected and related to epidemic patterns and outbreaks that have already occurred, and might be predicted to occur elsewhere. These variants could possibly lead to the development of appropriate vaccines and thus population based vaccination strategies. The aims of surveillance of this nature are to isolate and determine the strains circulating from sampling clinical cases, estimate morbidity and mortality, to establish prevalence of relevant antibodies and to guide the formulation of vaccines with an appropriate cocktail of antigens to protect against the dominant strains in circulation for a given year.

In the UK surveillance has been well developed through a network of diagnostic laboratories and dependable sources of epidemiological information (for example the Public Health Laboratory Service (PHLS)). Incidence of the virus is monitored by the weekly collection of data on deaths, sickness benefit claims, General Practitioner reports and laboratory confirmation of influenza virus reports (Pereira and Chakraverty (1982)). Together they have documented most epidemics since Asian 'flu' (H2N2) in 1975 (Chakraverty et al. (1986), Chakraverty, Cunningham and Pereira (1982), Tillett and Spencer (1982)). In the USA the equivalents are the Influenza Research Centre (IRC) in Houston, Texas, which was set up in 1974 (Couch et al. (1986)) and the Centre for Disease Control (CDC).

Since influenza can be either the primary or secondary cause of mortality there can be no direct association between the prevalence of influenza and the mortality caused by it. Initial health will be also a major factor in the level of influenza-induced mortality observed. It was these problems which led Stuart-Harris (1976) to comment that 'thus far it has been impossible to devise a case-mortality rate for influenza'. However, since mortality statistics in general are regular and predictable (Farr (1947)) it is possible to assess, to a degree, the impact of a specific influenza strain in terms of the mortality which is 'in excess' of the normal observed mortality over the period under consideration (Cliff (1986)). Serfling (1963) devised a regression model which, by using data from the previous 20 years (ignoring ap-

parent epidemics) gives an estimate of the predicted normal mortality. Any mortality in excess of this level can be attributed to deaths due to any influenza epidemic. It is important to distinguish between influenza mortality, the total number of individuals who die as a result of influenza infection, and the case-fatality rate, which represents the number of influenza cases which directly result in the death of the infected individual.

The factors found to be important when making a forecast for each coming winter are: the knowledge of which viruses have circulated in previous years; which of them have been found in the immediately preceding months in the southern hemisphere; and what proportion of the population has already been exposed to homologous strains and is therefore possibly immune to an attack (Pereira and Chakraverty (1977)).

There now follows a detailed summary of the major epidemics which occurred in Britain from the late 19th century up to 1985. More explicit details of the epidemics of 1973 through to 1989 can be found in chapter 3.

1890-1892.

Originating from Central Asia, there were three successive waves; June 1890 (with a 20-30% attack rate), 1891 and 1892. In 1890 24.7% of those affected were in the 20-40 year old age group, 36.2% were 40-60 and 22.4% were 60-80, this group containing the maximum number of case mortalities (Stuart-Harris, Schild and Oxford (1985)).

1918.

First isolated in West Africa, the epidemic of the earlier part of this year was characterised by the disproportionate mortality rate in the 20-30 year old age group, and by the attack rate of the younger age classes, with 10 year old individuals and younger experiencing 30-40% attack rates, as compared to the 10+ age groups which only had attack rates of 20-30%. Four months after the initial 1918 epidemic another epidemic occurred in Britain where the 20-40 year olds could be seen to suffer extensively with pneumatic complications, leading to a 50% case fatality rate. Approximately 44% of all influenza deaths during this epidemic occurred in the 20-45 year old age group, which also accounted for 37% of all pneumonia and bronchitis patients (Stuart-Harris, Schild and Oxford (1985)).

1932-1956 (H1N1).

This was the first virus to be typed, and overall the mortalities observed during this period was less than that seen in earlier years, most likely as a result of the introduction of sulphonamides in 1939 and antibiotics in 1945, which reduced the severity of secondary infections. However the general diminishment of mortality is at present partly unexplained. The age distribution of cases was much unchanged (Stuart-Harris, Schild and Oxford (1985)).

1957-1958 (H2N2).

In the eleven years of the circulation of the H2N2 virus strain there were three winters (1959-1960, 1963-1964 and 1966-1967) during which reported influenza incidence was at a very low level. The same pattern was observed over another eleven-year period with another three-winter period of low activity: 1970-71, 1973-74 and 1976-77. So similar were these patterns that it was thought that a new strain would appear in 1979 and start the epidemic process over again. However this new strain did not materialise (Chakraverty et al. (1986)).

Apparently starting in China, the virus swept to Britain and the USA where 5-15 year olds suffered maximum attack rates (the rate at which individuals acquire infection per head of population) of up to 50% (Langmuir (1961)), which are not dissimilar to those observed in the USA during the 1918 epidemic (Frost and Sydenstricher (1919)). Overall, however, there was only a 17% attack rate (Fry (1958)). Mortality peaked in October of 1957 in Britain (Martin (1958)), mostly in the 55+ age group. There was a significant recrudescence in June 1958 which led to a proportionally higher mortality rate in the older age groups (McDonald (1958)).

1959-1967 (H2N2).

The epidemic of 1959 showed a higher death rate than that of 1957. No further variations of the virus surface antigen were recorded until 1962. The last epidemic of the H2N2 strain occurred in January 1968 (Miller and Lee (1969)) which produced a larger number of cases in the elderly than had been seen for several years.

1968-1970 (H3N2).

Because it was first identified in Hong-Kong, this strain has always been referred to as 'Asian flu'. The initial epidemic (1968) generated a low number of mortalities, but during the winter of 1969-1970 a large outbreak occurred in Britain with excess mortality being observed, although in the USA excess mortality was noted during the 1968 epidemic as well (Assaad, Cockburn and Sundaresan (1973)). Mortality and morbidity had patterns similar to the H2N2 strain but on a lower scale, with the under fives having the highest attack rate and the 5-14 year olds the lowest; in adults the highest attack rate was in the 45-64 age group, and the lowest in the 65+, which was even lower during the 1968-1969 epidemic (possibly due to their having experienced similar strains during childhood). Why the discrepancy exists between the USA and Europe is not understood. A moderate epidemic occurred in the winter of 1971-1972 also due to A/Hong-Kong/68.

1971-1977 (H3N2).

During this period there were three active winters; 1971-1972 (due to A/Hong Kong/68); 1972-1973 (A/Eng/72); and 1975-1976 (A/Vic/75). The last of these strains gave rise to the largest epidemic and the highest mortality since 1969-1970, and is also the most divergent strain from A/Hong Kong/68 (Pereira and Chakraverty (1977)). There were only minor outbreaks in the years 1973-1974 and 1974-1975 which were caused by A/Port Chalmers with a minimal excess mortality. In the winter of 1977-78 there was a slight increase in deaths due to influenza A/Vic/75 and A/Texas/77 with the 25+ age group having experienced the H1N1 strain from the 1957 epidemic.

1978-1985 (H1N1).

Although this strain was typed as 'Russian flu' (A/USSR/77) the place of origin may well have been China. Attack rates in residential schools ranged from 20% to 80%. A great variation in clinical severity was observed, although it is considered that the epidemics had become less severe since 1976 (Sabin (1978)). Of those infected approximately 90% were under 25 years of age, which helps to explain the lack of excess mortality. The high prevalence in the younger age classes could be explained by the exposure of the older groups

to the H1N1 strains of the 1946 to 1957 epidemics (Chakraverty, Cunningham and Pereira (1982)). The epidemics of 1979-1980, 1980-1981, and 1984-1985 were all moderately intense and contained H1N1 and H3N2 in variable proportions. Waning mortality in Britain is most likely due to immunity conferred by the 'old' H3N2 strains, which have circulated since 1968, and probably induce protection against other H3N2 strains. A similar situation was found in 1950 with the H1N1 strain which has circulated since the 1940's.

1.4.2. Morbidity and mortality; the Pathogenicity of Influenza.

Relatively little is known concerning the precise pathological effects of the influenza viruses in man. Infection appears to be confined to the epithelium and sub-epithelial layer of the mucosa of the respiratory tract, but exactly where the primary lesion occurs is unknown. Destruction of epithelial cilia prevents efficient upward flow of the mucosa and necrosis of the epithelial cells themselves allows opportunistic pathogens to become established and disturbs the self-cleansing properties of the lungs. There is good reason to believe that it is this pathology which allows bacterial persistence and proliferation in the terminal air passages (Stuart-Harris, Schild and Oxford (1985)).

The virus has been recorded as having an attack rate of 33% of the population per annum (Couch et al. (1986)). Morbidity rates show that about 25% of all febrile respiratory illnesses are attributable to the influenza virus. Approximately 12% of the population seek medical help each year due to the illness, 0.12% are hospitalised and roughly 0.01% of the population dies from influenza each year (Couch et al. (1986)). Table 1.2. shows the hospitalisation and death rates for different age classes. From this data it would appear that the influenza virus leads to a majority of cases in the elderly and very young, and is most lethal in the elderly. It is important to note, however, that these groups are more likely to be hospitalised as a precaution against serious illness than individuals of middle age (in this table the 5-54 year old group). Table 1.3. shows the excess mortality associated with influenza A from chronic non-specific lung disease in England and Wales 1968-1976.

Kilbourne (1960) states that human viruses circulate at a relatively fixed level of virulence, the observed differences in severity of illnesses and mortality being due, in his opinion, to host variation. It has been observed that clinical influenza varies considerably in

Table 1.2. The estimated hospitalisation and Death Rates among individuals with acute respiratory disease during influenza epidemics; Houston 1978-1981 (data from Couch *et al.*).

Age	Hosp.Rate	Death Rate.
0-4	0.37%	0.003%
5-54	0.06%	0.002%
55-64	0.14%	0.015%
65+	0.42%	0.076%

Table 1.3. The Excess Mortality associated with Influenza A from chronic non-specific lung disease in England and Wales 1968-1976 (data from Stuart-Harris *et al.* (1985)).

Year	Total deaths*	Adj. deaths**[+]	Est. Excess mort.[+]
1968	33 292		
		21 109	1900
1969	34 262		
		22 806	4300
1970	29 856		
		17 788	0
1971	29 352		
		18 673	1600
1972	13 405		
		18 977	2600
1973	27 016		
		16 640	1000
1974	26 557		
		16 717	1700
1975	26 101		
		17 132	2900
1976	25 313		
Total	262 154	149 842	15 800
Annual average:	29 128	18 730	2000(+/-410)

* During 1 year

** Influenza season: October to April (inclusive)

+ Data for between-year season (Oct. to April).

severity from individual to individual depending on the age of the individual, the existing health of the individual as well as the virus strain (Cliff et al. (1986)).

Frank et al. (1985) also state that 'epidemiological differences appear to be more important than pathogenic potential in determining the community impact of these two subtypes [H1N1 and H3N2] of type A influenza virus'. Due to the fact that influenza induced deaths are extremely difficult to isolate (Stuart-Harris (1976)), it is not possible to determine whether specific strains are responsible for higher rates of cases, or fatalities, than others. However, variation in virus virulence has been suggested by several studies which record the temperature sensitivity of individual strains (Oxford, Corcoran and Schild (1980), Chu et al. (1982)). Also, the inability to replicate at temperatures greater than 38oC is a feature of virus strains which have been attenuated in virulence for possible use as vaccines (e.g. poliovirus). Oxford found that there was a higher proportion of viruses with this property among the A(H1N1) viruses recirculating from 1977 onwards than among other isolates such as those of subtype H3N2 (Oxford, Corcoran and Schild (1980)). Chu et al. (1982) also found a higher proportion of temperature-sensitive viruses amongst recirculating subtypes of a particular strain, namely H1N1. The suggestion that these findings indicate that the virulence of recirculating H1N1 viruses may be reduced is, however, not yet conclusively confirmed.

Variation towards increased virulence of natural influenza strains has not been observed in the past 55 years. The occasional outbreak with apparently greater severity such as that recorded by Nagler among Eskimos, was probably the result of a reduced past exposure of the residents because of geographical isolation (Nagler, van Rooyen and Sturdy (1949)). Thus, lack of exposure to any strains of influenza allowed the susceptible proportion of the population to increase, due to the recruitment of new-born individuals. Until the number of susceptible individuals rises above a certain level,the virus is unable to cause an epidemic. This concept , the 'threshold theory', is discussed in detail in chapter 2.

1.4.3. Biological features of infection and disease.

Over the past 50 years localised pandemics and epidemics of influenza have been consistent in their overall patterns. However, it has become apparent that there is considerable

variation in both the severity of illness in individuals and in the proportion of those individuals that develop complications. This is related to the virulence of the virus, the age at infection (Collins (1929)), the immunological status, determined by any previous experience to similar strains (Kilbourne (1960)), and to the health of the individual; the symptoms of influenza in people with a history of good health differ from those in ill health (Stuart-Harris et al. (1985)).

Although the clinical aspects of uncomplicated influenza in any one age group are similar, variations in incidence of certain symptoms do occur for different ages; for example, vomiting and convulsions only occur in infants; croup only in young children; sore throats and myalgia only in adults (Glezen (1980)).

Following direct droplet airborne transmission from infected individuals the incubation period is normally 48 hours, but may range from 24-96. Variation in the incubation period is dose dependent, with an abrupt onset of illness usually developing within one hour. Occasional occurrence of vague prodromal symptoms (such as coryza) before the onset of fever, tends to indicate that infection dose in these individuals may have been near to a threshold limit. Subclinical, asymptomatic infections can occur, possibly due to threshold infection doses, particularly in those with pre-existing serum antibodies (Thacker (1986)).

Symptoms in adults commonly include a marked fever, headache, photophobia, shivering, a dry cough, malaise, muscular ache and a dry, tickling throat. Fever is usually continuous, and classically lasts for three days at which point the temperature falls and symptoms abate. In some cases a second rise in temperature, smaller than the first, may occur after this time (Stuart-Harris et al. (1985)).

Of the acute symptoms listed above, the cough may persist for several days (leading to expectoration of mucoid or mucopurulent sputum), the eyes are often watery, burning and painful in movement and they can become blocked or give off a purulent discharge. Cervical adenopathy is unusual, but has been described and myalgia is most severe in the leg muscles but may also affect other extremities. Infection usually resolves itself in 7 days but infected individuals often feel unwell and listless after acute infection and can suffer from depression (Evans (1978)).

Complications can occur in the respiratory tract, the cardiovascular system or in the central nervous system, but the most commonly observed are respiratory related. These involve mostly the mucosa from the larynx to the alveoli, giving rise most commonly to simple tracheo-bronchitis. Opportunistic infections include secondary bacterial pneumonia, most often Streptococcal or Staphylococcal, generally in those with a history of chronic lung disease (Hers et al. (1958)).

There are two ways in which the cardiovascular system is concerned in relation to influenza. The first is the increased risk of pulmonary complications in patients with valvular heart disease (Hers et al. (1958)), and the second, more direct, evidence of cardiovascular complications is seen in infected individuals without previous cardiac disease who exhibit inflammatory myocarditis (Finland et al. (1945) and Martin et al. (1959)). There is however no reasonable explanation of the cardiovascular damage which must unquestionably occur in association with influenza and which may explain the risk of death in those with pre-existing cardiac disease who contract influenza.

During the Asian pandemic of 1957-1958 (see part 1.4.) a large number of neurological illnesses of varied nature occurred in many different countries. Flewett classified these illnesses into fatal encephalitis, post-influenzal encephalitis and cases of Guillain-Barre syndrome (Flewett and Hoult (1958)). However, it should be noted that there is no direct histological evidence to link the influenza virus with encephalopathy, which is itself a relatively common childhood condition, nor is live influenza virus necessary for provocation of Guillain-Barre syndrome (Stuart-Harris, Schild and Oxford (1985)).

1.5. Immunology.

1.5.1. Immunity to Influenza.

On current evidence, it appears likely that antibodies to the haemagglutinin and neuraminidase proteins confer protection against challenge from homologous virus strains and subtypes, with antibodies directed against the haemagglutinin being the more important of the two. The anti-haemagglutinin antibody neutralises virus infectivity and inhibits the primary infection, whereas the anti-neuraminidase restricts dissemination of the virus within

infected individuals (and thus the shedding of virus from the respiratory tract) and reduces the severity of the illness. There is no evidence to show that the matrix or nucleoprotein stimulate significant amounts of antibody production in infected individuals (Couch and Kasel (1983)).

There is not always a clear-cut relationship between the concentration of antibody in the blood and resistance to infection (Tyrrell et al. (1981)). This is obviously an important consideration in this study, both in terms of serologically derived results and in the mathematical modelling of immunity. However, in the absence of evidence to the contrary, it is assumed that the presence of antibody is indicative of immunity.

In response to haemagglutinin proteins IgM, IgA and IgG antibodies appear simultaneously within two weeks of inoculation. IgM and IgA peak at two weeks and then decline, whereas IgG continues to increase towards a maximum titre at four to seven weeks. IgM and IgG antibodies occur in all infected individuals but IgA only occurs in about 50%. Analysis based on nasal secretions reveal similar temporal patterns, but with IgM and IgG in lower concentration and with IgA found in all infected individuals. Observations based on antibodies directed against the neuraminidase proteins shows essentially the same patterns but there is far less documentation on the antibody production to this antigen.

All antibody production shows an initial fall over six months and then stabilises for two to three years. There can be a two- or ten-fold drop in antibody levels over this period, but it would seem that the serum antibodies persist for many years (Couch and Kasel (1983)).

Mouse models have indicated that macrophages may be responsible for virus clearance from infected areas of the mucosal tissue, while cytotoxic T-cells appear to cause a reduction in the amount of virus present in the lungs (McMichael (1982)).

1.5.2. Cross-reactivity.

Antigenic similarities between the surface antigens haemagglutinin and neuraminidase of influenza A viruses of different subtypes have been observed even in the absence of demonstrable cross-reactions in various immunodiagnostic tests (for example Immuno double

diffusion). These include evidence of relationships based on cross-protection or of cell-mediated immunity (Stuart-Harris, Schild and Oxford (1985)).

Both animal and human sera fail to exhibit cross-reactivity between haemagglutinin or neuraminidase antigens of different subtypes (Couch and Kasel (1983)). The multiple antigenic determinants expressed by the different strains lead to the phenomenon of 'Original antigenic sin' (where exposure to new strains elicits the production of antibodies to strains that have been previously experienced) (Webster et al. (1982)). Cross-reactivity, however is a commonplace occurrence between strains within a particular subtype (Oxford et al. (1980)).

Antibody production to strain-specific antigenic determinants occurs in higher frequency among persons experiencing natural infection than in those vaccinated with attenuated vaccines. It can be said that antibodies induced by exposure to the influenza virus are mainly directed to determinants shared by different subtypes. This is because haemagglutinin has multiple variants, only a few of which are altered in different strains. Since the total antibody response consists of the total combined antibodies of differing specificity, a number of changes are required before little or no cross-reactivity is observed (Couch and Kasel (1983)).

Therefore it can be expected that in infections with related strains of the same subtype there will be some proportion of the antibodies directed against one strain that will confer some immunity to other, closely related, strains.

1.6. Serological Methods.

Serological epidemiology has proved to be of great value in the study of several viral infections and associated diseases (both the course of infections in individuals and the pattern of incidence of infection in host populations (White and Fenner (1986) and Evans (1982a)); most notably poliomyelitis, measles, mumps, rubella and various arthropod-borne diseases (Black (1959)). Case to case household studies, combined with clinical studies of the course of infection, have led to the quantitative measurement of various stages of infection such as the latent, infectious and incubation periods as well as the levels and duration of specific

antibodies. In large scale population surveys the age-related changes in the proportion of the population possessing antibodies to the virus can be ascertained. Information of this type can lead to insights into the age and time-related incidence of infection, the levels of immunity to infection and, where relevant, the effects of vaccination programmes on these levels. When considering the dynamics of a virus in a population it is important to understand the concept of herd immunity. This principle is based on the belief that the chance of acquiring infection by a virus within a community is related to both the density of susceptibles and the density of infectious individuals. This concept, as well as that of immunity due to co-circulation of similar virus strains is dealt with fully in later chapters.

There have, in recent years, been rapid developments in quantitative epidemiology with the use of mathematical models and statistical methods, which have been used to study transmission dynamics and the control of directly transmitted viral diseases, such as those mentioned earlier, in many countries (Dietz (1976), Knox (1980), Anderson and Grenfell (1986) Anderson and May (1983a, 1983b, 1985), Grenfell and Anderson (1985), Nokes, Anderson and Anderson (1986), Anderson et al. (1987), McLean and Anderson (1988) and Nokes and Anderson (1988)). To make accurate assessments and predictions from studies of this sort it is essential that the data which is collected must be reliable and authentic. Data can be obtained from two major sources: case notifications and serological studies.

Case notifications can be unreliable for a number of reasons, one of which is that asymptomatic infection (or very slight infection) is common in many diseases (including mumps, rubella and influenza) and may therefore not be reported (Feldman (1982)). Another reason is that there is often a bias in reporting cases in the younger age groups (via concerned parents) and in the older age groups (where the infected individuals' health is more at risk due to complications). It is also probable that infections will be more often reported during school term than during holidays or before school age (Black (1982)). Finally, and with great relevance to the present study, influenza is not officially a notifiable disease in the United Kingdom so only the acutely ill cases will tend to be reported. Case notification data have however been used quite extensively to represent age-specific acquisition of infection and can be used in the absence of suitable serological data to give an estimation of epidemio-

logical parameters and patterns (Anderson and Grenfell (1986), Anderson and May (1983a, 1983b, 1985), Grenfell and Anderson (1985) and McLean and Anderson (1988)).

Most serological studies to date have made use of pre-existing collections of sera from particular communities. These originate from a variety of sources (e.g. blood transfusion centres and hospitals) and are seldom collected on a random basis, but more commonly are drawn from particular spatial areas or age groups in a specific community. Ideally the sera should be composed of age-stratified samples taken at random from males and females in a defined population, and should represent all socio-economic classes. Most serum sets described in past published work (Chakraverty, Cunningham and Pereira (1982), Chakraverty et al. (1986), Pereira and Chakraverty (1982) and Pereira and Chakraverty (1977)) are limited in the extent to which they meet these criteria (typically limited age ranges and small overall sample size are the major restriction).

Until recently, methods of defining humoral immune response among infected humans were limited. Epidemiological research was based on results from the use of conventional complement-fixation (CF), haemagglutination-inhibition (HI) or neutralization (Nt) tests for antibody in sera before or after infection. However the recognition of heavy (11S) IgA secreted at the mucosal level (see section 4) led to studies of antibody secretion using conventional methods (Couch and Kasel (1983)).

The serological response to influenza vaccines is usually assessed using HI or neuraminidase-inhibiting (NI) antibody tests. These tests are widely used in a standardised format, which are easy to perform and give reliable, reproducible results. However HI tests are relatively insensitive and do not detect low levels of antibody (Jennings, Smith and Potter (1981)).

Recently, new serological tests have been used for the estimation of influenza antibody, including single radial haemolysis (SRH), single radial diffusion (SRD) and radioimmunoprecipitation (RI). Another recently developed technique, the enzyme-linked immunosorbent assay (ELISA) has been used for the detection of antibody to several viruses, including mumps, measles, rubella, respiratory syncytial virus, herpes simplex virus, varicella zoster virus as well as the influenza virus. In most of these studies ELISA has proved to be con-

siderably more sensitive for the detection of antibodies than other techniques (Koskinen et al.(1987), Julkunen et al. (1984), Julkunen et al. (1985), Van Voris et al. (1985)).

1.6.1. Seroepidemiology of Influenza.

Antibody surveys have been conducted for the influenza virus for the years 1977 to 1985 (Periera and Chakraverty (1982) and Chakraverty et al. (1986)) using the haemagglutination inhibition technique. Although these surveys are not reported in detail it is obvious that the proportion of the population with antibody to the circulating viruses of that time is considerable in all age groups. It is suggested that the high levels of antibody in the adult population generated against the H1N1 subtypes found in the late 1970's are a result of exposure to this subtype during the previous decade of prevalence, 1947-1957. The levels of antibody to the influenza H1N1 subtype in persons under 20 increase noticeably between 1977 and 1980. The proportion of the population with antibody to the H3N2 subtype is consistently higher in all age groups than that with antibody to the H1N1 subtype. This is presumably due to the more recent exposure to the virus.

More relevant to this study are the levels of antibody generated against the A/Eng/333/80 (H1N1) and A/Bel/1/81 (H3N2) strains in the mid 1980's. In this case the overall levels of antibody to the H1N1 subtype are higher than those for the H3N2 subtype in general, but there are higher antibody levels in the young age groups against the H3N2 subtype. An increase can be seen in the proportion of persons over 50 years of age who are seropositive to the H3N2 subtype, whereas no similar increase can be seen in the proportions seropositive to the H1N1 subtype.

A more thorough study was performed by Stuart-Harris et al. (1981) considering the proportion of the population immune to the A/Hong Kong/68 strain (which is also considered in detail in this thesis) for the time period 1968 to 1970. The results of this study clearly indicate a declining in the proportion immune through time.

1.7. Transmission of the virus.

For the purposes of this study it is assumed that, like measles, mumps and rubella, influenza virus is transmitted directly, via airborne droplets from infected individuals to susceptible individuals. This is referred to as direct transmission. It is also assumed that the incidence of influenza infection in a population is a function of the number of susceptibles and the prevalence of infectious cases, which is equivalent to the 'law of mass-action' first proposed by Hamer in 1906 (Fine and Clarkson (1982)).

It is the contention of Hope-Simpson (1979) that the influenza A virus, after infecting its host, remains latent in many individuals for a period of time. Due to various seasonal phenomena, caused by 'variations in solar radiation', the viruses emerge simultaneously from many latent infections, with altered surface antigens, to cause fresh epidemics (Hope-Simpson and Golubev (1987)).

Hoyle, after studying influenza epidemics in schools, cast doubt on the direct transmission theory and proposed that influenza viral precursors must be reaching earth from an extra-terrestrial source (Hoyle and Wickramasinghe (1990)).

However, neither hypothesis has found general recognition, nor been widely accepted, amongst other academics in this field.

1.8. Modelling virus transmission.

The interactions among co-circulating strains of related viruses have received little attention in the theoretical literature (Castillo-Chavez et al. (1987) and (1988) and Selby (1976)). Until recently little information was available with regard to level and duration of cross-immunity, but recent studies (Couch and Kasel (1983)) show a considerable degree of long-lasting cross-immunity between related strains of human influenza. Through cross-immunity, the presence of one strain of the virus can reduce the pool of susceptible individuals for co-circulating strains and thereby reduce the potential for survival of those strains.

The type of model presented here is a deterministic compartmental model which describes the change in age-prevalence of disease that takes place over the course of time. Such

models have already been extensively studied for other diseases, and have been usefully applied, for example in answer to questions about optimal vaccination policies in developed countries (for example Anderson and May (1985) and Schenzle (1985)). However, existing models have always considered the population either to be at an endemic equilibrium, or to be altering in response to perturbations by vaccination. The question of cross-immunity has not been explored except as a mathematical concept (Castillo-Chavez et al. (1987) and Castillo-Chavez et al. (1988)).

The model used throughout this thesis is based on the result of expressing, in mathematical terms, what are believed to be the major biological processes that determine the epidemiology of influenza in a developed country (i.e. one with a comparatively large, stable population). The major objective of this study is to gain a greater understanding of some of the processes or interactions which produce the patterns of epidemics and pandemics which are observed for the influenza virus.

A large part of the work in reaching these goals lies in the development of methods of data analysis. These allow the interpretation of both the previously existing epidemiological data (such as case notifications) and also that data which has been made available due to the serological studies described in another part of this thesis. The data facilitates the derivation of parameter estimates for inclusion into the model. Provided with a set of initial parameters, the properties of the model can be explored under different conditions by numerical methods.

Since the earliest recorded application of calculus to disease transmission by Daniel Bernoulli, who presented his results on the impact of inoculation against smallpox to the Acadamie Royale des Sciences in 1760, considerable advances have been made in this field. Investigation of disease transmission is now conducted though two major media; stochastic or deterministic models.

Stochastic models describe the probability of one new case occurring in a given unit of time, as compared to deterministic models which describe the number of new cases which occur in a given time period. Stochastic models are generally used for small populations such as households, where 'chance effects' are of great importance. Most stochastic models

are derived from chains of binomial distributions used to describe the number of cases incident in successive generations of the disease. Reed and Frost lectured with these types of models in the 1930's, but did not publish any of their work until 1976 (Reed and Frost (1976)). Since then Becker has introduced the concept of heterogeneity in infectiousness of individuals (Becker (1981)), and the heterogeneity of contact rates, within and between households, has been considered by Becker and Angulo (1981). Stochastic models are most useful in estimating the duration of various stages in the disease such as the lengths of the incubation and infectious periods, and can be used to estimate transmission rates (Elveback et al. (1968)). Other than these uses, stochastic models are limited in the context of larger communities.

Oscillations in incidence of infectious diseases are widely observed phenomena within human communities. Often the periodic or epidemic outbreaks of infection are very regular in occurrence, as exhibited by the influenza virus. Such patterns are of considerable interest to both epidemiologists and mathematicians. It is now widely accepted that non-seasonal fluctuations arise as a consequence of the dynamic interactions between two or more populations. Long-term studies of viral and bacterial diseases in mice colonies show that fluctuations in incidence were largely determined by rate of influx of susceptible animals and the acquisition of immunity (Greenwood et al. (1936)). Mathematical evidence comes from Lotka (1923), Kermack and McKendrick (1927) and Soper (1929), with stochastic work done by Bartlett (1956).

It is hoped that, by using the mathematical principles outlined above, it will be possible to determine the major factors which cause the influenza virus to follow the dynamic patterns of transmission which are observed.

1.9. Aims of the investigation.

The primary objective of this thesis is to gather epidemiological data on the interactions of co-existing strains. It is intended that this data will provide a basis for exploring the factors that determine the transmission dynamics of the strains under study using simple mathematical models.

For this purpose case notifications and serological horizontal cross-sections are analysed to discover any trends in the transmission of influenza virus strains, both between age groups of the population and during the time period for which the strains were maintained in the population.

The thesis is organised as follows. The following chapter details a mathematical model which describes the transmission dynamics of two co-existing virus strains in a population without age-stratification. The model serves to highlight the principle factors that control transmission within human communities.

Chapter 3 describes analysis of data on notifications of cases of influenza to the Communicable Disease Surveillance Centre. The data are considered with respect to time (longitudinal studies) and age (horizontal studies), to dissect age and time related trends in transmission.

Chapter 4 describes the methods used to devise a suitable laboratory-based serological test to determine the presence or absence of antibody directed against the specific surface antigens of each strain of the influenza virus considered.

In chapter 5 these results are analysed to determine the proportions of different age classes in the population which are immune to the various strains under consideration at the time of sera collection. The data thus allows the examination of any temporal or age-related trends in the acquisition of infection.

The penultimate chapter uses the parameters obtained from chapters 4 and 5 in the mathematical treatment of the theoretical concepts which have been mentioned earlier, and are detailed in chapters 2 and 6, to consider the epidemiological forces behind the observed changes in the immune status of an age-structured population.

Finally, chapter 7 compares the observed trends, arising from the analyses in chapters 4 and 5, with the patterns derived from theoretical concepts explored in chapters 2 and 6. Any anomalies arising from these comparisons are discussed and explanations are proposed.

This is the first time that both the analysis of observed data and theoretical concepts involving the transmission dynamics of co-existing strains of the same virus in a human popu-

lation have been presented simultaneously. As a consequence some areas are not dealt with in complete detail. However, it is hoped that this thesis serves as a useful foundation for further work on the subject.

Chapter Two: Mathematical Modelling.

2.1. Introduction.

In this chapter the mathematical model used in the study of influenza virus transmission is described. The chapter begins by defining the compartments of the host population which represent susceptibility, infection and immunity, and setting down the equations used to determine the flow of individuals between these compartments. The parameters which control the transitions between states are defined and their properties summarised. The basic model does not take account of any heterogeneity in the population with respect to age. Its behaviour is analysed both in terms of the equilibrium states of the model and by numerical methods. Chapter 6 considers the more realistic (and hence more complex) situation of an age-structured population in which heterogeneous mixing between age classes is assumed.

The equilibrium states of the model are used to facilitate data interpretation and to express various epidemiological parameters in terms of quantities that can be measured from recorded epidemiological data of various kinds.

2.1.1. Basic Principles of Mathematical Epidemiology.

The origin of modern theoretical epidemiology owes much to the work of several late 19th and early 20th century scientists, the most prominent of whom include Farr and Brownlee, who described the courses of various epidemics in terms of frequency distributions (Farr (1840) and Brownlee (1907), and Hamer and Ross, who were the first to consider the problems associated with the transmission of infectious diseases in terms of simple but precise mathematical statements (Hamer (1906) and Ross (1908)).

The notion that the course of an epidemic depends on the rate of contact between susceptible and infected individuals was first proposed by Hamer in (1906). This theory, now called the 'mass action principle', assumes that the net rate of spread of infection is proportional to the product of the density of the susceptible population multiplied by the density of the infectious population. This concept was translated into a continuous-time model by Ross during his work on the transmission dynamics of malaria (Ross (1911)).

Following detailed study of the mass-action theory, Soper (1929) deduced the underlying mechanisms responsible for the periodicity of epidemics (namely fluctuations in the density of susceptible individuals, caused by demographic and epidemiological processes), while Kermack and McKendrick (1927) established the 'threshold theory'. The threshold theory states that an epidemic of an infectious agent will not occur unless the infectious individuals are introduced into a population of susceptible individuals the density of which is above a critical level. These two tenets, the law of mass-action and the threshold theory, are considered to be the fundamentals of modern theoretical epidemiology.

Much of the work presented over the past three decades has considered the importance of variation and the elements of chance in the spread and persistence of infection, thus leading to the development of probabilistic (or stochastic) models (e.g Bartlett (1956) and Bailey (1975)), which are widely used in modelling influenza (e.g. Elveback et al. (1964)).

The areas of mathematical epidemiology which excite most interest at the present time include the application of control theory to epidemic models, the spatial spread of diseases, the mechanisms underlying recurrent epidemic behaviour, heterogeneity in transmission, and the extension of the threshold theory to encompass more complex deterministic and stochastic models.

2.1.2. The Epidemiological Modelling of Influenza.

The various types of influenza models considered here can be conveniently categorised by the size of the populations involved; these range from small family groups to the populations of whole continents. In accord with this disparity of scale the models used contain a diverse number of concepts, some far more complex than others. In this section it is intended to give an overview of the problems involved in the modelling of influenza, and thus to explain the rational behind the choice of model employed in the remainder of the chapter. Since influenza is used in this thesis only as an example of a virus with co-existing strains the previously published models dealing with influenza are not explored in great detail.

The models most often applied in studies of intra-familial contagion are the 'chain-binomial' models. These discrete time-step models consider the spread of infection in a fam-

ily as a series of binomial trials; each exposed susceptible individual has a certain probability of becoming infected each time a new case occurs in the 'family'. A derivation of the simple chain binomial was proposed by Reed and Frost (1976) which takes into account the probable number of cases that an individual will be exposed to during the average interval of infectiousness of an infected person (this probability changes during the course of the familial epidemic). Details of both models (in mathematical terms) can be found in the review by Bailey (1976).

The main advantage of studying the transmission of an infectious agent through small communities (i.e. 50 to 5,000 individuals) are that the characteristics of the individuals can be detailed; i.e. their susceptibility can be assessed, and significant amounts of information can be gathered for use in the model with regards to mixing patterns. Two types of model are used in conjunction with this type of data, assuming either random or non-random mixing populations. The best known of these small community influenza models is that proposed by Elveback et al. (1964). The model, designed to consider a non-homogenous, non-randomly mixing human population of 1000 individuals, is fully stochastic. Individuals which make up the model population pass through the various stages of disease (detailed in section 2.2.1) in a random fashion, thus conferring unique characteristics, with regard to the course of disease, to each individual. The populations used were designed to mimic, as closely as possible, an American community, with each member of the community having characteristics assigned to them on separate coded input cards. These characteristics even included social and behavioral aspects. This model has been used extensively to test, amongst other concepts, that of the secondary attack rate (SAR) as an indicator of transmission rates (Longini et. al (1984)).

Most epidemiological investigations of influenza are based on morbidity or mortality estimates from large populations (Fine (1981)), which are notoriously imprecise due to the error and bias of the notification system (see also chapter 3). Deterministic models are generally used, since chance contact becomes of negligible importance in very large populations (i.e. 10 to 50 million individuals), and transmission chains are essentially hidden. Large population models, therefore, are most frequently deterministic, mass-action formulations of random-mixing, homogenous populations. The long-term simulation of influenza infec-

tion in large populations introduces many problems, associated with the persistence of the virus and the observed seasonal epidemics. There is a recognised tendency for influenza epidemics to have a 'right-skew' shape over the course of time, and attempts to model this (using the deterministic formulation mentioned above) have yielded varying degrees of success (Spicer (1979)). In general the model is fitted to observed mortality data by a method of least squares, with a fixed mortality rate. It is interesting to note that Spicer's application of the mass-action model excluded data which gave bimodal epidemics. In the light of the observation (in chapter 3) that most epidemics of influenza consist of two, co-existing, strains which lead to the appearance of a bimodal epidemic this omission is a serious one.

Much work has been performed on the geographical spread of the influenza virus in the USSR, and an appraisal of the work (by Baroyan and Rvachev) is reported in the review by Fine (1981). More detailed analysis of this model is given by Rvachev and Longini (1985). The basic model is an inter-locking system of deterministic, mass-action equations describing the transmission of the virus within and between 128 geographical regions (each of which is described by a separate set of continuous-time mass-action equations). The geographical regions include urban and non-urban areas, each with a specific rate of transmission for the virus. Rate of transfer between these regions is designed to represent the immigration and emigration of the individuals themselves.

The final use of mathematics in the epidemiological study of the influenza virus is concerned with the concept of 'excess mortality'. This is a relatively simple idea and involves the regression, in some form or other (commonly by the method of least squares), of a function (consisting of sinusoidal components) to observed mortality data. By estimating the changes in the average mortality, in the absence of influenza-induced mortality, over a number of years it is possible to recognise any departures from the expected trends (Serfling (1963)). The usefulness of these types of 'predictive' models are limited, in that they predict no epidemics, and simply analyse past events to ascertain temporal trends in the severity of morbidity and mortality.

All the models described above, however, have some limitations. The chain binomials, when applied to familial data (Hope-Simpson (1979)), showed that there was marginal evi-

dence for intra-familial transmission. Although flexible to a degree, the Reed-Frost model of Elveback et al. (1964) gives no potential for generalisation for larger populations. Direct mass-action models have mostly relied on morbidity or mortality estimates for the generation of numerical results. As such these predicted patterns have been compared to observed morbidity or mortality trends.

As a consequence of the previous research on the transmission dynamics of the influenza virus described, and the observed age-dependant variation in the transmission parameters (see chapters 3 and 5) it was decided to use a compartmental, deterministic mass-action model, considering two interacting strains of the virus and incorporating (in part II) non-random, heterogenous mixing with respect to age. The model was compartmentalised to take into account the various stages associated with the spread of the influenza virus through a large population. These compartments, and the assumptions made in deriving the model, are described in the following section.

It is hoped that the model used in this thesis will go some way to clarify the problems associated with the dynamics of influenza, including those problems raised in earlier chapters, such as the lack of recurrence of specific strains, the bi-modal properties of many epidemics and, in the light of an apparent 'fade-out' of virus in the non-seasonal months, the peculiarity of consistently high levels of seropositivity after the introduction of a particular strain.

2.1.3. The Course of Infection with the Influenza Virus.

The course of infection with the influenza virus has been summarised in chapter 1, but since it is of considerable importance with respect to compartmental models, the various stages associated with infection are considered in detail here. Infection with the virus does not necessarily imply that an individual will contract the disease. A significant number of individuals become infected but do not manifest the disease clinically. Since these asymptomatic individuals are infective, they are important in determining epidemiological patterns. Therefore infection with the influenza virus must be specifically distinguished from clinical influenza, the disease.
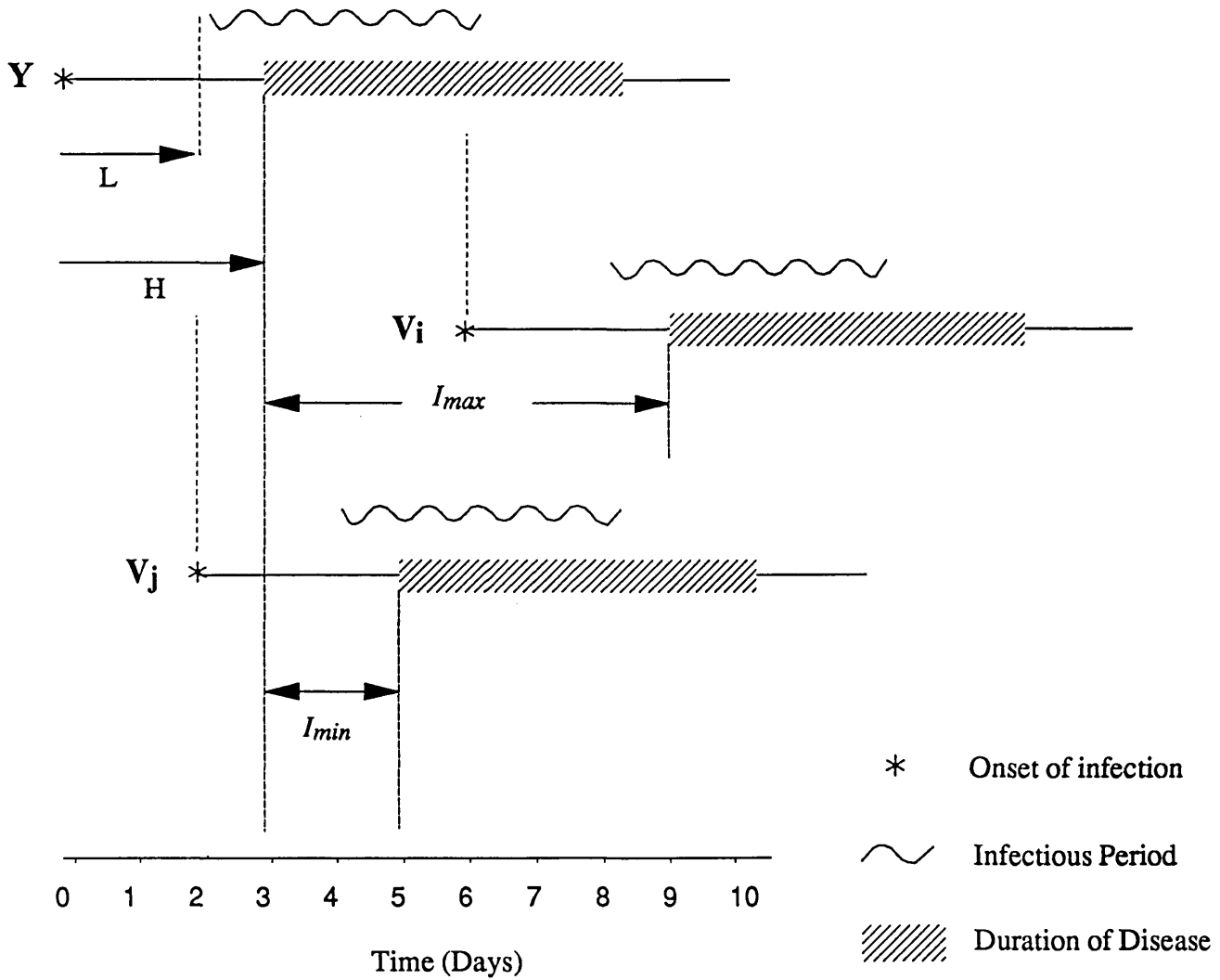
Figure 2.1; The course of infection with two strains of the influenza virus. **Y** *represents the first virus strain, where* **Vi** *and* **Vj** *represent the second strain.* I_{max} *is the maximum interval between two infections, and* I_{min} *is the minimum time. L is the latent period and H is the incubation time. (Adapted from Fine (1981)).*

The period of time from the point of infection to the appearance of symptomatic disease is defined as the 'incubation period' (Hope Simpson (1948)), which is considered to be between 24 and 72 hours (Sartwell (1950)). The duration of disease itself, if apparent, can greatly affect the morbidity statistics and is therefore of some epidemiological importance. The duration of the disease, clinical influenza, is approximately 2 to 7 days, with an average of 6 days (Evans (1978)).

Of greater significance to the transmission dynamics of the virus than the parameters discussed above are the 'latent period' and the 'duration of infectiousness' (see Fig. 2.1). The period of time between an individual becoming infected with the virus and the initiation of shedding viral particles (i.e. having the potential to infect other individuals) is termed the latent period. The latent period is considered to be slightly shorter than the incubation period, but some argue that it may in fact be significantly longer (Hope-Simpson (1979)). The duration for which an individual is infectious is somewhere of the order of 3 to 6 days (Evans (1978)), and has considerable implications on the dynamics of infection, with respect to the time interval between infections with viral strains (see Fig 2.1).

For the purposes of the model detailed in the next section two strains of influenza are considered to co-exist, and it is assumed that individuals are susceptible to both strains of influenza virus when they are born. Because of the deterministic nature of the system, all susceptible individuals become infected with one or other of the virus strains during the course of the life of the individual. It is assumed that life-long immunity to one infective strain is conferred on the infected individual after infection with that strain. In addition, in this system, a certain proportion of all individuals infected with one strain become immune to the second strain, and thus cannot become infected by this second strain (the biological evidence for these assumptions is due to the 'shift' and 'drift' of the antigenic determinant of the influenza virus, described in chapter 1). There are therefore two levels of immunity: it is possible to be immune to one strain, but not the other; or immune to both strains.

The mathematical model described in the remainder of this chapter attempts to encapsulate these stages of infection in a biologically realistic manner. The subpopulations described are represented by different compartments, with the flow of individuals between

them dependant on the epidemiological parameters determined by a combination of factors relating to the biology of the virus strains, to the mixing patterns of the population and to the durations of each phase of infection.

2.2. The Model.

2.2.1. An Introduction to the Model.

The model used throughout is compartmental and deterministic, and is derived from that described by Castillo-Chavez (1988). In chapter 6 the model is expanded to include age structure and heterogenous transmission rates.

Essentially, the model consists of a set of linked differential equations describing the flow between subsets of the population, with initial and boundary conditions for these equations. The population is divided into five groups; those individuals who are susceptible to infection from either strain (designated as the sub-population X); those who are infectious with one of the strains but not the other (sub-populations Y_1 or Y_2, depending on which strain); those who are immune to infection from this strain, but not the other (sub-populations Z_1 and Z_2); those who are infectious with a second strain, having recovered from an infection with the other (V_1 or V_2 depending on the strain of current infection); and finally those individuals who have been infected with, and are therefore immune to, both strains (sub-population W). Figure 2.2 shows the compartments of the model and the directions of the change in the sub-populations.

A number of assumptions are inherent in the definition these classes. Firstly, it is assumed that no protection is conferred upon infants by maternal antibodies. This is contrary to most deterministic models of this kind which deal with endemic childhood diseases. It is assumed in this model that the whole population has not previously been exposed to the strain of virus which is epidemic. Secondly, no 'latent' class is used (unlike most models) which intimates that most infected individuals are instantly infectious. No influenza-induced mortality is considered, since the relative numbers of mortalities due to the virus are negligible compared to the population size (50 million). Finally, it is assumed that antibody protection is lifelong, a fact suggested by the results reported in section 5.4.2.
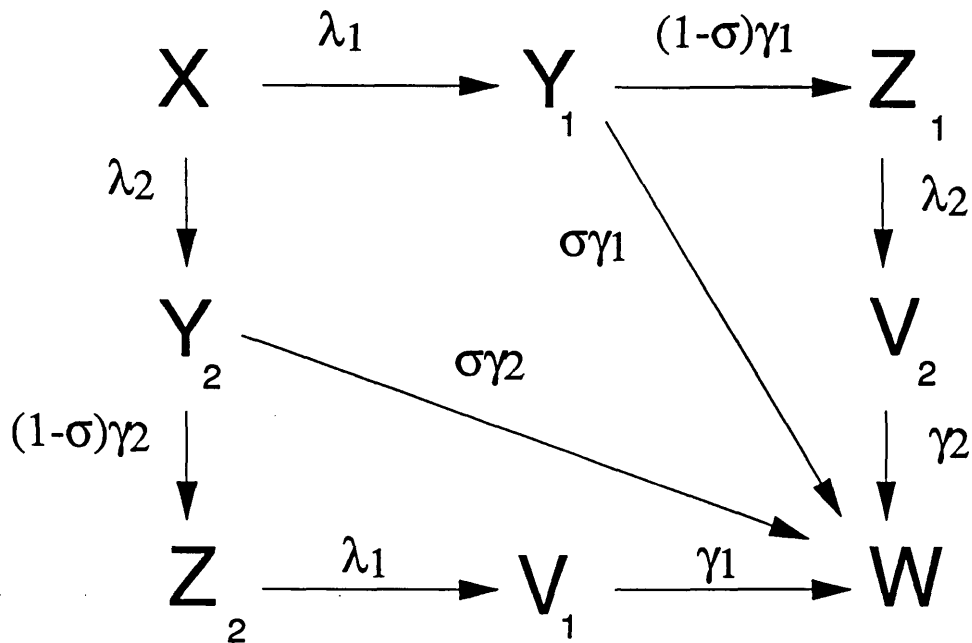
Figure 2.2; Schematic flow diagram of the compartmental model for the transmission dynamics of two co-existing strains of virus; 1 and 2. X represents the susceptible population; Y those individuals infected with one virus; Z those immune to one strain only; while V represents the individuals infected by two strains consecutively; and W represents those people immune to both strains of the virus. The rate parameters are λ the force of infection, γ the recovery rate and σ the cross-immunity coefficient (see text for details).

This model of the dynamics of the influenza virus is original in that it differs from that of Castillo-Chavez in one major respect. Instead of assuming that cross-immunity simply regulates the flow of individuals from the first immune class to the second infectious class, it considers that a proportion of those infected initially by one strain will become immune to both strains, while the remainder will only be immune to that strain. To facilitate the mathematical description of this system, immunity is considered to be complete in the selected proportion of the population, preventing any infection with the second strain. In terms of bi-

ological reality, this may not be entirely accurate, but it is not possible to represent partial immunity in some individuals with the framework defined in Fig. 2.2.

As mentioned above, these five classes (or compartments) are represented by the following letters, where i can be 1 or 2, representing the strain. Thus if i represents strain 1, j, representing strain 2, must be equal to 2.

X = Susceptible;

Y_i = infected by only one strain (strain i);

Z_i = immune to only one strain (strain i);

V_i = infected by a second strain (strain i) after the first (strain j);

W = Immune to both strains.

The following are the Ordinary differential equations which represent the changes from one class to another.

$$dX/dt = \mu N - [\beta_1(Y_1+V_1)+\beta_2(Y_2+V_2)+\mu]X \qquad (2.1)$$

$$dY_i/dt = \beta_i(Y_i+V_i)X-(\gamma_i+\mu)Y_i \qquad (2.2)$$

$$dZ_i/dt = (1-\sigma)\gamma_i Y_i - [\beta_j(Y_j+V_j)+\mu)]Z_i \qquad (2.3)$$

$$dV_i/dt = \beta_i(Y_i+V_i)Z_j-(\gamma_i+\mu)V_i \qquad (2.4)$$

$$dW/dt = \gamma_1(\sigma Y_1+V_1)+\gamma_2(\sigma Y_2+V_2)-\mu W \qquad (2.5)$$

$$N = X + Y_1 + Y_2 + Z_1 + Z_2 + V_1 + V_2 + W.$$

where N = population size.

The parameters involved in the model are; μ the per capita mortality rate, where the average life expectancy is $1/\mu$ (measured in years); σ, the cross immunity coefficient, represents the degree of herd immunity conferred on a community to infection with a second strain of the virus as a result of previous exposure, of the population, to an antigenically similar strain of the same virus during an earlier season. The value of the cross immunity coefficient is measured as a proportion of the population.

βi, the average number of 'successful' contacts (i.e. those contacts resulting in a successful transmission of the virus), per infective, per day (the daily contact rate) for the virus strain i; and γi, the daily recovery rate, where $1/\gamma i$ is the infectious period in days for a person infected with strain i (see Fig. 2.1). The infectious period is set such that the values are equal for both strains 1 and 2 since there is no significant biological evidence to the contrary. These parameters lead to some useful expressions in terms of the rate of change of the population; γY represents the rate at which individuals recover from infection and $\beta i(Yi+Vi)$ denotes the per susceptible rate, or force, of infection with virus strain i.

2.2.2. Parameters involved in the model.

The parameter values are estimated from various sources including epidemiological data (see chapters 3 and 5), clinical observations and demographic tables. The magnitude of the parameters determines the 'rates of change' between the various classes associated with infection. It is the parameter assignments, therefore, that facilitate the comparison of observed situations with predictions in terms of the models behaviour under controlled circumstances. In this study, where possible, the parameters have been determined from the epidemiological data available. The parameters under consideration are summarised in Table 2.1.

It is important to reiterate that throughout this chapter it is assumed that all parameters are constant with respect to both age and time.

Table 2.1; The base-line parameters used in the numerical analysis of the non age-structured model.

Parameter	Symbol	Value
Time Step	t	1 day
Death rate	μ	3.7×10^{-5} day^{-1}
Recovery Rate	γi	0.16 day^{-1}
Proportionality Constant	βi	0.4 day^{-1}
Cross-immunity Coefficient	σ	0.5

(a) The Constant of Proportionality (β)

The constant of proportionality, β ,is the combination of two biological quantities; the rate of contact between infectious and susceptible individuals per unit of time; and the probability that a contact will result in infection. In this chapter β is assumed to be a constant with respect to time and age, and is measured per person per day. In this case, where a constant proportionality is used for all ages, the values are estimated to give biologically realistic numerical analyses. During the numerical analysis of the model the value of the proportionality constant for strain 1 is held constant at the base-line value, and the value for strain 2 is varied. This allows an examination of the model under controlled conditions of parameter variation.

In chapter 6 the constant of proportionality is expanded to cover the situation where β i s heterogenous between age groups; the age- dependent function $\beta_{(a,a')}$ is discussed, and the concept of the 'who acquires infection from whom' (WAIFW) matrix is introduced (Anderson and May (1984)).

(b) The Cross-immunity coefficient.

For the purposes of this study it is assumed that a certain proportion of those persons infected with either strain 1 or 2, in the first instance, will become immune to both strains. This proportion is represented by the parameter σ; the cross-immunity coefficient. This parameter is determined from observed data by calculating the ratio of persons infected with strain 1 compared to those infected with strain 2 when the two strains are epidemic in the same season (see chapter 3). The value of σ is assumed to be a constant value between 0 and 1 for the interaction between two co-existing strains, but to differ between varying pairs of strains. The actual estimation of this parameter is somewhat problematical, since it is not possible to determine which strains confer immunity on other strains. However, by considering strains which co-exist in the same season (although not strictly speaking causing immunity between each strain) and from other data of this sort (Castillo-Chavez (1988)) it is possible to derive crude values for σ.

(c) The Recovery rate (γ).

The recovery rate, γ, is determined from the duration of infectiousness, and is considered to be the reciprocal of this time period, which is estimated as being between 3 and 9 days (Evans (1978). This gives a value for γ of between 0.33 and 0.17 day^{-1}. For the majority of the numerical analyses the recovery rate is considered to be equal for both strains of the virus, since there is no evidence to the contrary at present. However, this value is varied for the second strain for some analyses, to determine the effect that this has on the dynamics of the two strains. While the value for the recovery rate is varied for strain 2, it remains constant at the base-line value for strain 1.

(d) The Mortality rate (μ).

The mortality rate is determined as the reciprocal of the average life expectancy, taken to be 75 years throughout this study, giving a value for μ which is 0.0133 year^{-1}. Obviously this constant mortality rate leads to the somewhat unrealistic result of some individuals living to an age considerably in excess of 75. However, due to the relatively short time period over which the model is run, this simplification does not have a major impact on the predicted trends in the incidence of infection. In the age-structured model presented in chapter 6 the value of this parameter is of more consequence, and therefore a more realistic step function is used to represent the host mortality (Anderson and May (1983)).

As mentioned earlier, no disease-induced mortality is considered.

2.3. Equilibrium Properties of the model.

An important concept, introduced by McDonald (1952) and refined by Dietz (1975) and others, is that of the 'reproductive rate' of an infection, R_0. For directly transmitted viral infections R_0 is defined as the average number of secondary infections produced when one infectious individual is introduced into a population of susceptible individuals of defined density (this is equivalent to the 'net reproductive value' of Fisher (1930)). Therefore, R_0 depends both on biological factors that control the course of infection in an individual host and on environmental (or social) factors relating to contact within the host population.

For a virus which is at an endemic equilibrium in the host population it is possible to determine the fraction of the total population still susceptible to infection from the virus (Anderson and May (1991)). This is denoted by $\bar{x}$, where

$$\bar{x} = X/N.$$

For a homogenously mixing population, $\bar{x}$ is related to the value of R_0 as follows:

$$R_0 = 1/\bar{x}$$

(Anderson and May (1991)). In this situation, where two strains of the same virus coexist, it is possible to consider two separate values for Ro. The basic reproductive rate of the virus itself (taking into account the effects of both strains on the susceptible population) can be represented as R_0', and is not easily determined. However, the basic reproductive rate for each of the strains of the virus in (R_{0i}') can be determined using equation (2.7). To give an indication of the values of the basic reproductive rate, these values are calculated for the separate strains, ignoring the effects which will be caused due to the co-existence of these strains.

Table 2.2; Observed and Predicted values for the Basic Reproductive Rate for two co-existing strains, assuming all parameters are as detailed in Table 2.1, except the proprtionality constant for the second strain, which is varied. The model is run until equilibrium is reached.

	Reproductive Rate (R_0)	Proportionality Constant (day^{-1})	x
Observed: [+]	8 - 20	-	-
Predicted: [*]	2.5	0.2	0.40
	3.1	0.4	0.32
	4.2	0.6	0.24
	5.3	0.8	0.19

[*]Estimated from equation (2.7).

[+]Data from table 5.2 using equation (6.18)

This in effect assumes that R_{0i}' is equivalent to R_{0i} (the reproductive rate for the virus in isolation), which is probably incorrect. Unfortunately, it is beyond the scope of this thesis to calculate values for the strain-specific basic reproductive rate in the situation observed (R_{0i}'), where cross-immunity affects the strain-specific values of R_{0i}, by limiting the fraction of the population which is susceptible to infection with other, co-existing virus strains.

The number of susceptibles, X, and the total population, N, are as defined earlier. Obvious limitations are placed on the generation of secondary cases of infection when the fraction of the population susceptible becomes less than 1, assuming that the acquisition of infection is directly proportional to the density of the susceptible population (the assumption of 'weak homogeneous mixing'(Anderson and May (1985)). If there are an unlimited supply of susceptible individuals the number of secondary cases of infection is simply the value of R_0. However, if some fraction of the population is immune, the resultant number of infected persons will be dependent on both the fraction of the population susceptible and the basic reproductive rate of the virus strain, R_{0i}. This introduces the concept of an 'effective' reproductive rate, R_i (Anderson and May (1983b)), which is defined as

$$R_i = R_{0i}\bar{x} \qquad (2.6)$$

By definition, an infective agent at an endemic equilibrium in a host population will, on average, produce exactly one secondary infection from every primary infection. Therefore, at equilibrium, the effective reproductive rate will always be equal to 1, and hence

$$R_{0i}.\bar{x} = 1. \qquad (2.7)$$

It is, therefore, an easy task to obtain an estimate for the strain-specific basic reproductive rate, R_{0i}, from the serological data presented in chapter 5, bearing in mind that these values probably do not represent the situation observed here exactly since the strains are not at an endemic equilibrium, however, this is the best estimate that can be made. It is also possible to compare these with estimates made from the numerical analysis of the model (see Table 2.2).

Other methods commonly used to estimate R_0, from equilibrium versions of equations similar to those presented here, but representing the transmission dynamics of only one strain

(e.g. Anderson and May (1983) and Dietz (1975)), are not applicable to this system due to the complexity of the equations under study. Studies on single strain systems have shown a relationship between the rate parameters and the value of R_0, which is reliant on the assumption of 'strong homogeneous mixing' (i.e. the overall transmission of the virus is dependant on both the density of the susceptible and infected individuals). However, for the case involving two co-existing strains described here, the infectious population is derived from two separate sources; those individuals infected with only one strain and those infected consecutively with both strains (the Y and V populations respectively in equations (2.1)-(2.5)). This introduces sufficient complexities in the derivation of an expression for $\overline{x}$ in terms of the rate parameters that the resultant solution to the equations does not provide a simple definition of R_{0i} (which would effectively be Roi'; the reproductive rate for a strain which co-exists with other strains, all dependent on the same pool of susceptible individuals). From table 2.2 it can be seen that much higher strain-specific basic reproductive rates are observed than are predicted by this model. From numerical analysis of the model, varying the constant of proportionality (which is equivalent to the transmission probability), it can be seen that this implies that the resultant equilibrium population of susceptible individuals should be smaller. This in turn may suggest that the values used for the constant of proportionality are too small in magnitude. However, from the results of the next section it can be seen that the presence of cross-immunity acts to lower the resultant equilibrium number of susceptible individuals, and thus correspondingly raises the value for the reproductive rate if the simple definition of Roi (based on a single strain of the virus) is used as defined in equation (2.7).

2.4. Numerical analysis of the homogenous mixing model.

The numerical analysis of the model defined by equations (2.1)-(2.5) was performed via integration using the fourth order Runge-Kutta method, with a fixed time step, via the differential equation solving software Solver (Rev. 4).

Using the 'base-line' parameters which have been determined (chapter 3), and estimates from the literature (see Table 2.1), and varying these parameters in a controlled fashion, it is possible to examine the changes in the sub-populations of the model under different con-
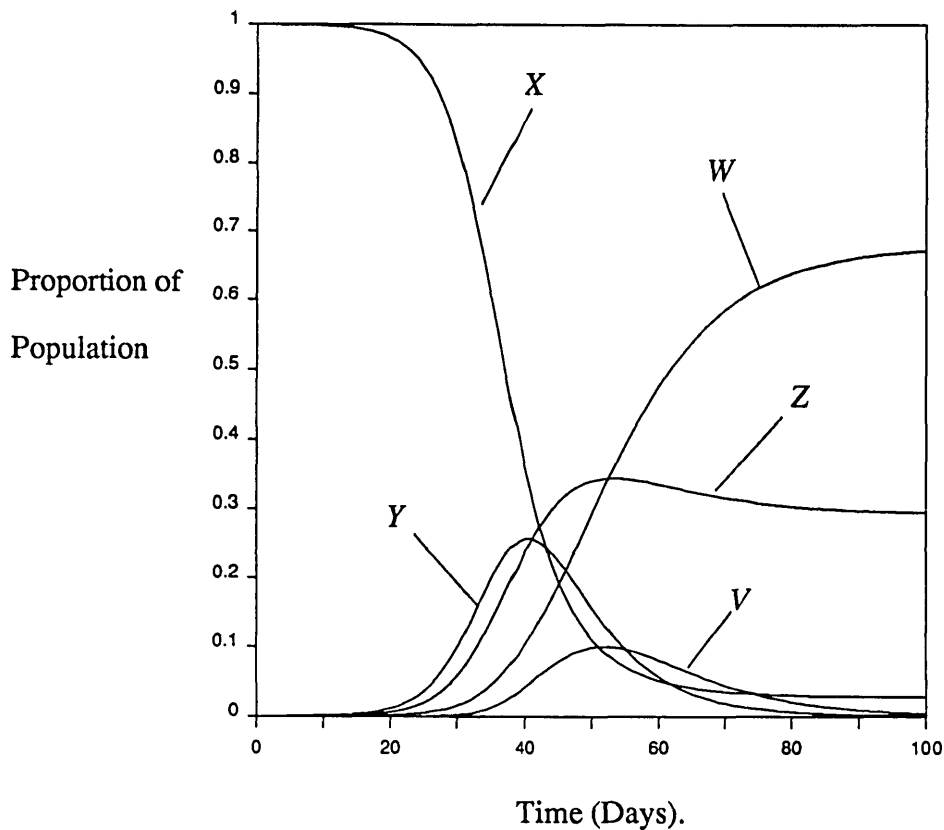
Figure 2.3; Numerical solution of the model using 'base-line' parameter values estimated from serological studies and demographic tables (see Table 2.1 for details); x is the proportion of the population susceptible, y and v are the proportion of the population infected with one or two strains respectively, z is that proportion of the population immune to one strain only, while w represents the proportion immune to both strains of virus.

ditions. A numerical solution of the non age-structured model over time, using the base-line parameter set is presented in Fig. 2.2 with all parameter values shown in Table 2.1. It is useful to compare the resultant epidemic with that described by the case notifications shown in Figs. 3.1 and 3.2, and to note the sharp increase in the proportion of the population which becomes seropositive. In both cases, the numerical analysis of the model and the observed case notification data, the epidemics are short-lived and, therefore, lead to a very rapid conversion of the population from susceptible to infected, and hence immune. It is this rapid seroconversion of the population which characterises the transmission of the influenza virus, and is, therefore, an important consideration in the mathematical treatment of this problem.

2.4.1. Variation in the proportionality constant.

Figures 2.4(a) and (b) show the response of the model to variations in the proportionality constants ($\beta 1$ and $\beta 2$) of the two strains. Perhaps the most important point which arises from this analysis is that the changes in the proportionality constant (which are within biologically realistic limits) do not effectively change the rate of rise in the proportion of the population which becomes seropositive, but merely limit the magnitude of the resultant proportions as time progresses.

Variation in the magnitude of the proportionality constant leads to some predictable results in terms of the size and shape of the epidemic curves (Fig. 2.4(b)). In this example, the value for $\beta 1$ is held constant at 0.4 day-1, and $\beta 2$ is varied between 0.2 and 1.0 day-1. It can be seen that in the situation where the two co-existing strains do not confer immunity to each other, there is a simple relationship between the size of the second epidemic and the magnitude of the respective force of infection. However, if there is an element of cross-immunity, the two strains compete for the pool of susceptible individuals. The strain which has the greater force of infection will therefore cause an epidemic of cases considerably greater than the strain with the lesser force of infection. This is illustrated in Fig. 2.5(a) and (b) which demonstrates the relationship between the strain specific force of infection of the second strain (where the transmission probability of the first strain, $\beta 1$, is kept constant at 0.4 day-1) for the two scenarios, with or without cross-immunity. It can be seen that when no cross-immunity occurs the number of individuals infected with the first strain is not affected by the magnitude of the proportionality constant of the second strain (Fig 2.5(a)). In the case where immunity is conferred on the second strain by the first, and vice-versa, it can be seen that the strain which has a greater 'infectivity' (as determined by the constant of proportionality) will be in a position to infect the pool of susceptibles significantly quicker than the strain with the lesser infectivity, and hence convert these susceptibles to non-susceptible individuals in terms of infection with the competing strain. Thus the strain with the larger value of Roi will have its reproductive potential reduced due to competition despite the fact that no change in the actual value of the proportionality constant occurs (Fig 2.5(b)).

Time (Days).
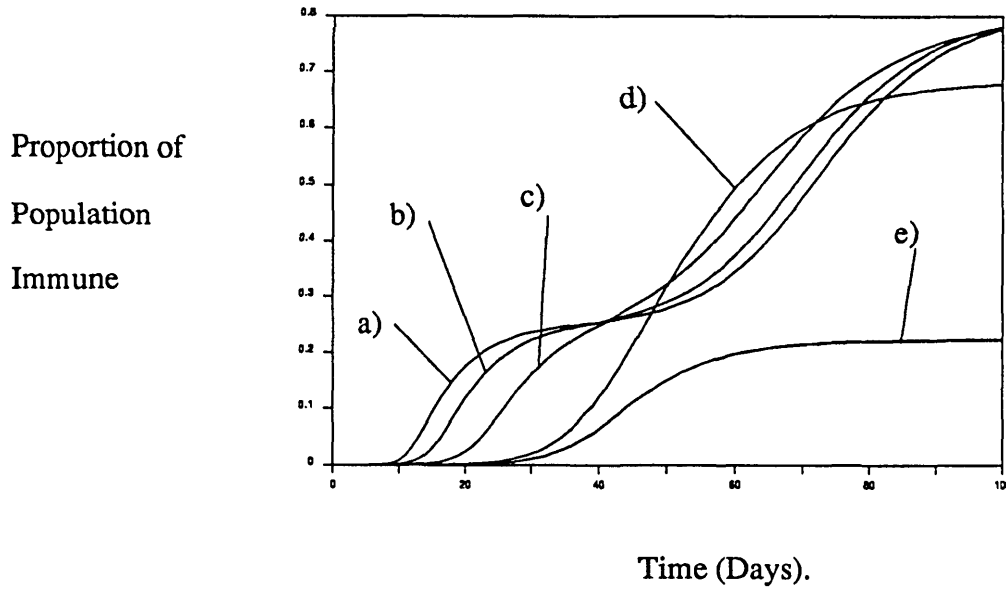
Figure 2.4(a); Variation of the proportion of the population immune to both strains; and (b) variation in the proportion of the population infected with the two strains, dependant on the varying proportionality constant; a) β_2 = 1.0, b) β_2= 0.8, c) β_2 = 0.6, d) β_2= 0.4, e) β_2 = 0.2 day^{-1}. The cross-immunity coefficient (σ) is 0.5 throughout, β_1 = 0.4 day^{-1}.

Time (Days)
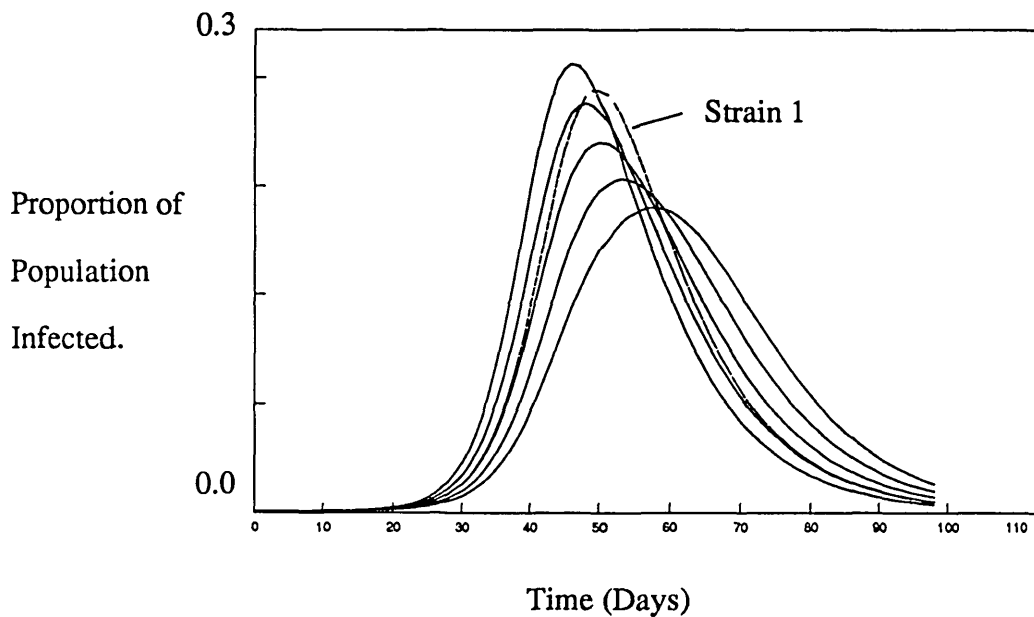
Figure 2.4(b); variation in the proportion of the population infected with the two strains, dependant on the varying proportionality constant; the dotted line represents those individuals infected with strain 1, and the other lines show the proportion infected with strain 2, where the top line is β_2 = 0.42, and the lower lines are β_2 = 0.41, 0.40, 0.39 and 0.38 respectively and β_1 = 0.4 day^{-1}.

Proportion of
Population
Infected

Proportionality constant for strain 2; β_2.

Proportion of
Population
Infected

Proportionality constant for strain 2; β_2.
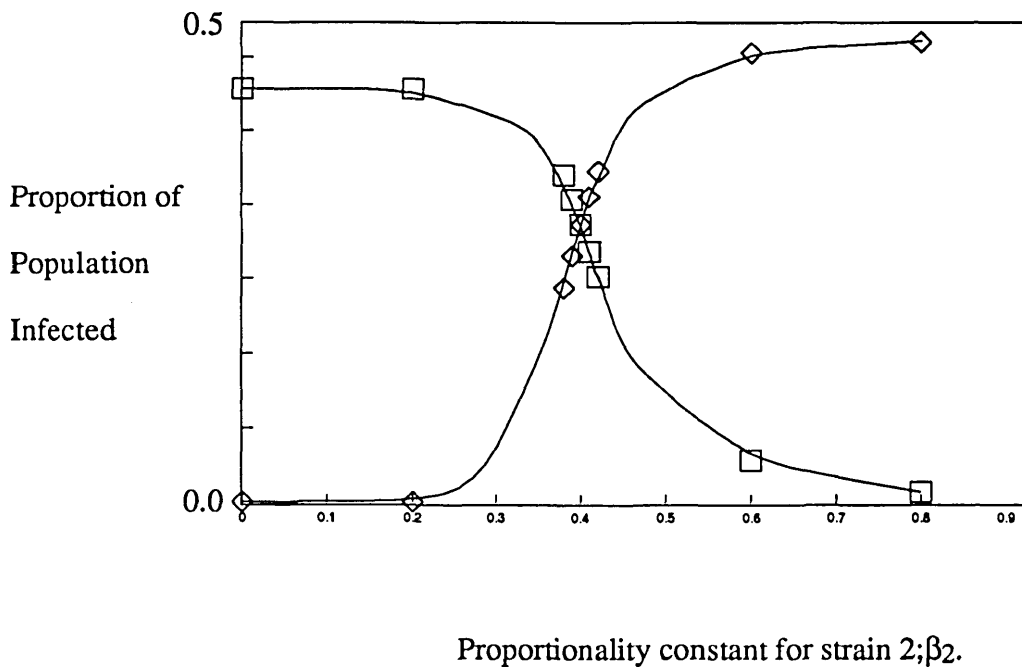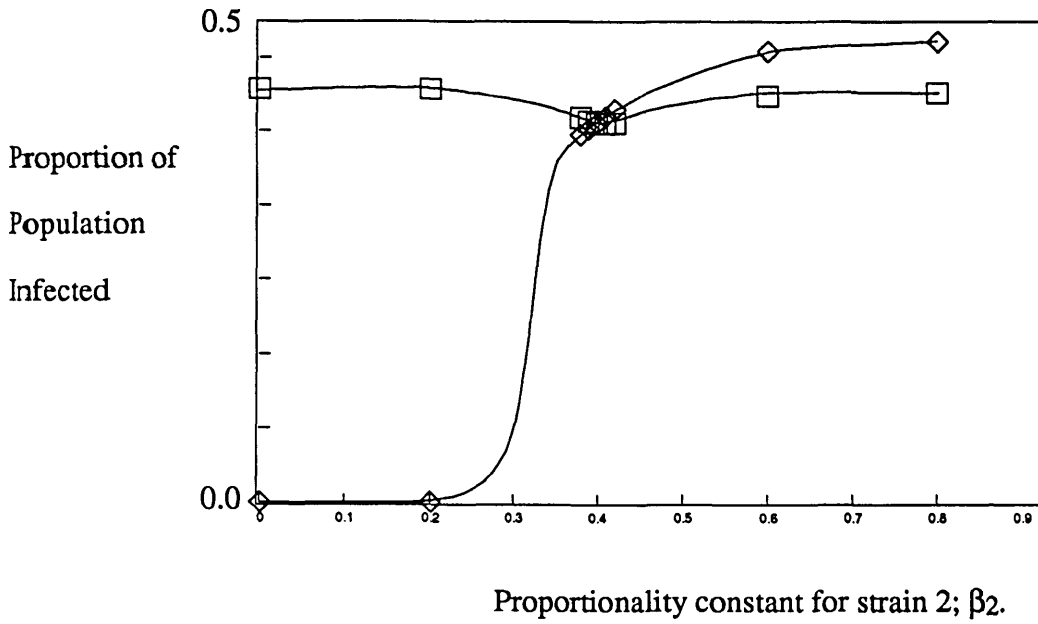
Figure 2.5; A comparison of the numbers of individuals infected with the two strains of the virus, and the variation in the proportionality constant of the second strain. All other parameters are the base-line parameters shown in Table 2.1, with $\beta_1 = 0.4$, and β_2 as shown. a) Shows the situation with no cross immunity, and b) shows that with a cross-immunity coefficient of 0.5.

1.0

Proportion of

Population

Susceptible '

Proportion of

Population

Infected.

0.0

Time (Days).
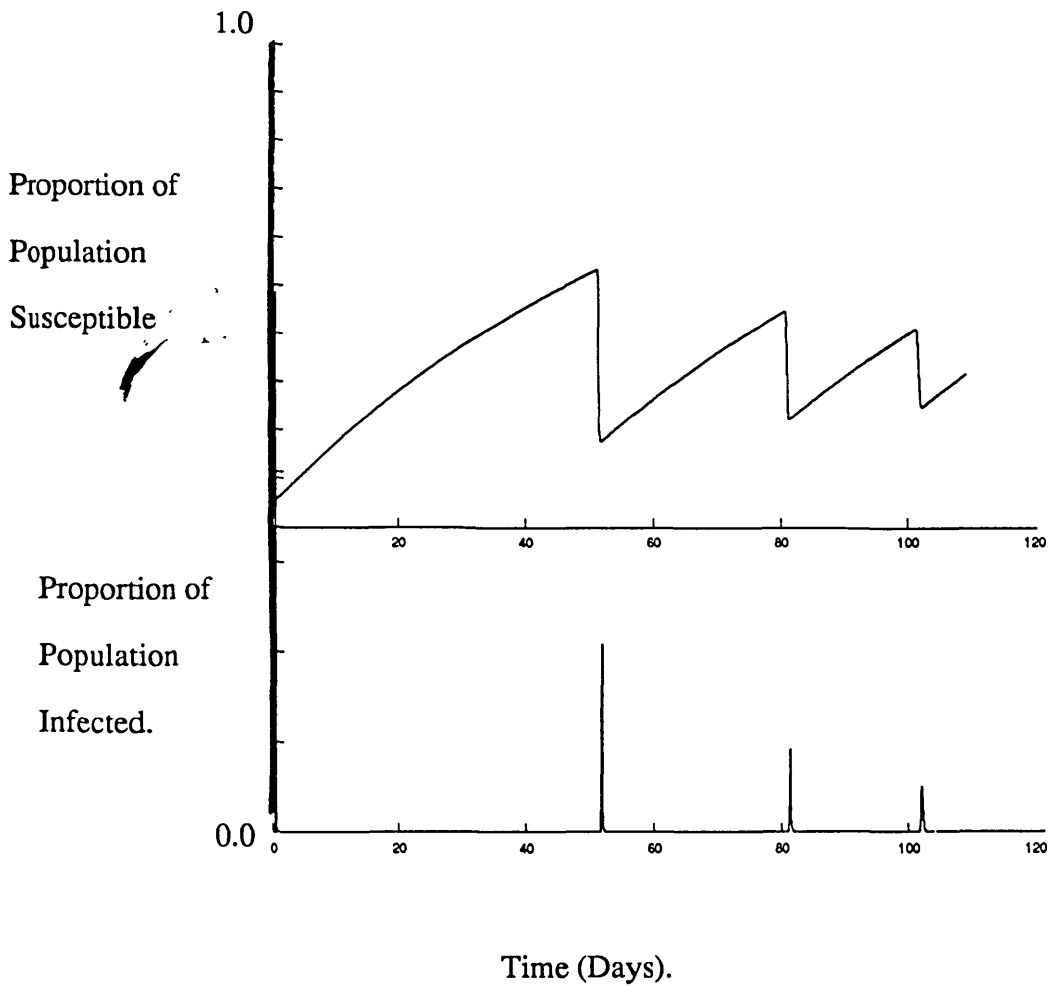
Figure 2.6; The results from numerical analysis of the model, with only one strain present (i.e. all parameters for the two strains are identical, as shown in table 2.1), over a period of approximately 110 years. Note the regularity of the recurrent epidemics, and the damped oscillations of the infected and susceptible populations.

1.0

Proportion of

Population

Susceptible

20   40   60   80   100   120

Strain 1

Proportion of

Population

Infected.

0   20   40   60   80   100   120

0.0

Strain 2

0   20   40   60   80   100   120

Time (Days).
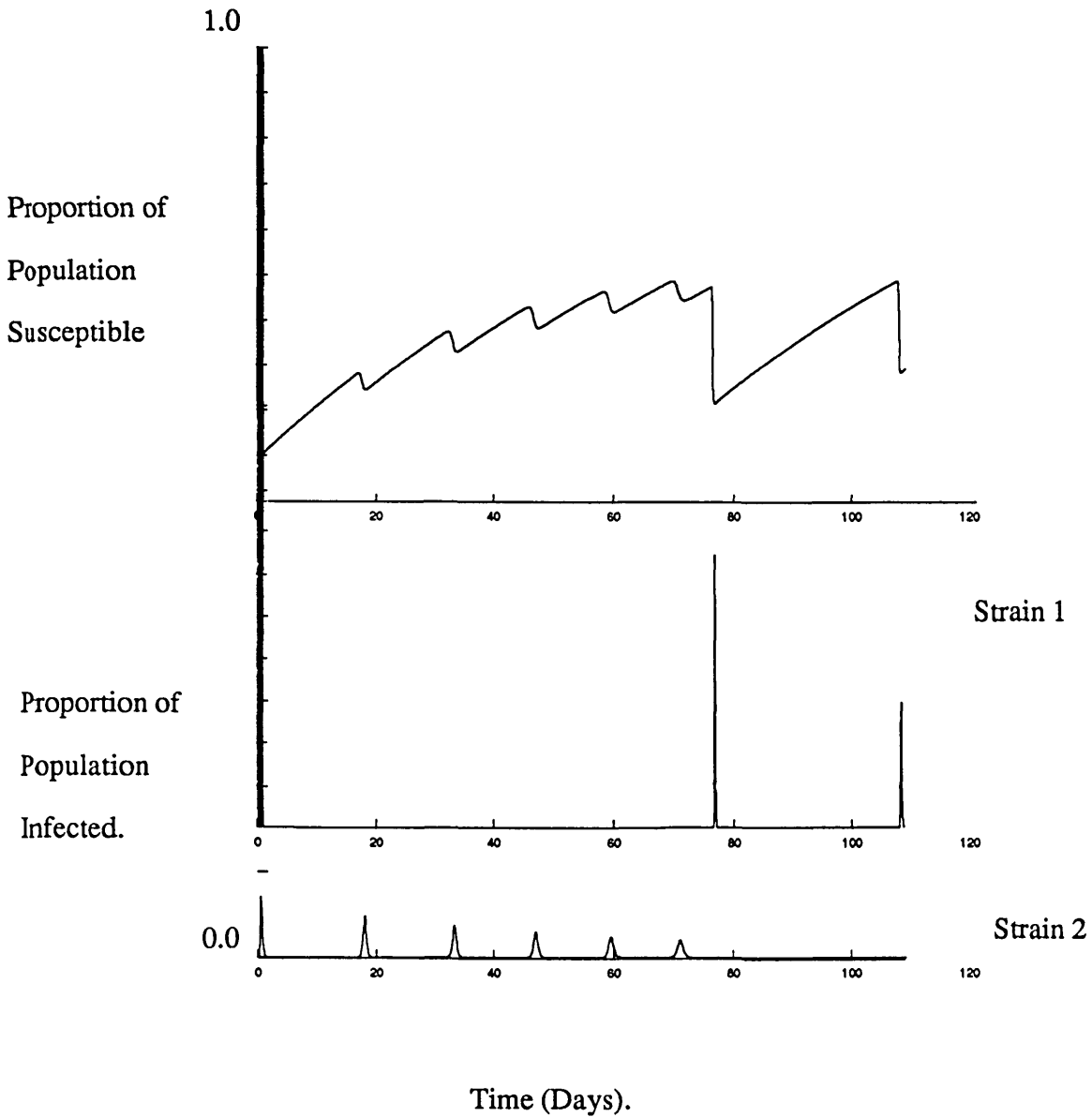
Figure 2.7; The results from numerical analysis of the model, with two strains present (the proportionality constants for the two strains are β_1=0.4and β_2=0.3day^{-1} respectively) over a period of approximately 110 years. All other parameters are as detailed in Table 2.1. Note that the susceptible population does not show classic damped oscillations, and the recurrence of the epidemics, considering both strains, is irregular.

In the long term (that is more than 50 years) the relative forces of infection are also important, in that with the equivalent of only one strain circulating (i.e. the forces of infection are the same) a system of damped oscillations is observed (Fig. 2.6). However, in the situation where the two strains have different forces of infection, each strain appears to be epidemic on a different time scale to the other, thus causing out of phase damped oscillations (see Fig. 2.7). This observation has considerable significance with respect to the observed, apparently unpredictable, epidemic nature of co-existing influenza strains.

It is also possible to introduce the second strain into the population after a time delay. Figure 2.8 shows the case where the second strain is introduced into the population 5 years after the first. No cross immunity is considered so that the epidemics caused by the two strains can be directly compared. It can be seen that no significant difference in the epidemic patterns are caused by this time delay. It can reasonably be assumed, therefore, that the same effects as those described above occur after a time delay between the introduction of the two strains. Therefore, it is possible to conclude that the effects of cross-immunity will be apparent in the same season or in temporally separate seasons.

Although the concept of the basic reproductive rate is dealt with in detail in chapter 6, it is useful to examine the effect of varying the proportionality constant on the value of the basic reproductive rate. Table 2.2 clearly shows that the reproductive rate is increased proportionally to the transmission probability (β_i) of the virus strains.

2.4.2. Variation in the cross-immunity coefficient.

The effect of varying the cross-immunity coefficient σ on the size and shape of the epidemics is shown in Fig. 2.9. From analyses of this type it is possible to derive a direct relationship between the value of σ as defined in section 2.2.2.c and the ratio of the proportion of individuals infected with the first strain compared to the proportion of individuals infected with the second. This relationship is shown in Fig. 2.10, thus, if it was possible to be sure which strain caused immunity to another it would be a simple matter to quantify this value, by determining the numbers of individuals infected by each strain of the virus.

By performing numerical analyses with varying cross-immunity coefficients it is possible to determine the effect of this parameter on the final equilibrium value of the sus-
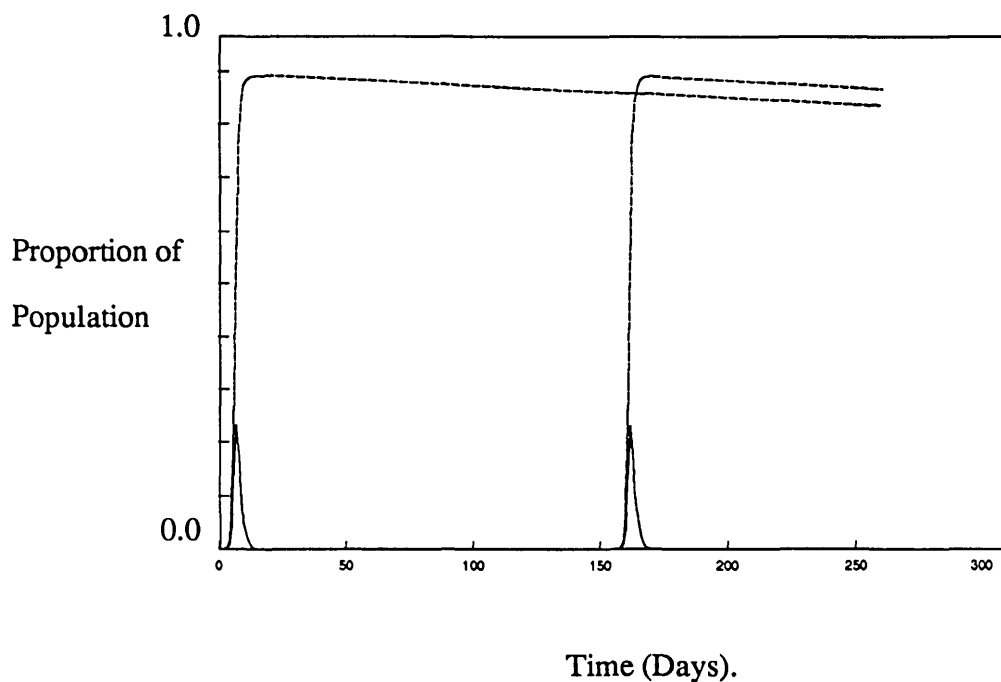
Figure 2.8; The results from numerical analysis of the model, with two strains present (the proportionality constants for both strains are $\beta_1=\beta_2=0.4\ day^{-1}$), *with the second strain being introduced 5 years after the first. Cross-immunity is assumed to be negligible, and it can be seen that the two strains are thus unaffected by each other, and the passage of time has little effect on the size and shape of the epidemic curve.*

Proportion of

Population

Time (Days).
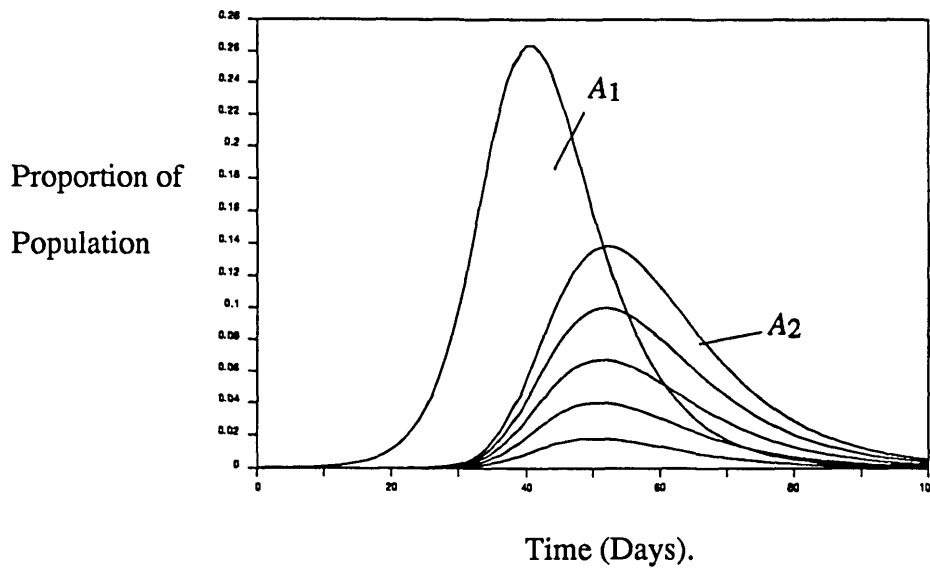
Figure 2.9; The effect of varying the cross-immunity coefficient (σ) on the number of individuals infected with the second virus strain. σ= 0.0 (top line of second strain), 0.2, 0.4, 0.6, 0.8, 1.0 (bottom line, which is on the x-axis). β is 0.4 day^{-1} throughout for both strains. A1 represents the total number of individuals infected with strain 1, where A2 is the (varying) number of individuals infected with the second strain.

ceptible proportion of the population and hence on the value of the basic reproductive rate of the virus strain. From analyses of this type it was found that, with a constant value for β of 0.8 day-1, the proportion of the population which was susceptible at equilibrium with a value of 0.0 for the cross-immunity coefficient (i.e. no cross immunity) was 0.45. If the cross-immunity coefficient was 0.8, this proportion was reduced to 0.365. These susceptible proportions of the population are equivalent to R_{oi} values of 2.22 and 2.74 respectively. Thus cross immunity between co-existing strains of the same virus would appear to raise the value of the joint basic reproductive rate (for both strains combined), Ro', over that persisting for either strain in isolation, Roi.

2.4.3. Variation of other parameters.

The numerical results derived from variation of parameters representing the infectious period and the average life expectancy are identical to those performed in similar studies on

Cross-immunity Coefficient (σ).
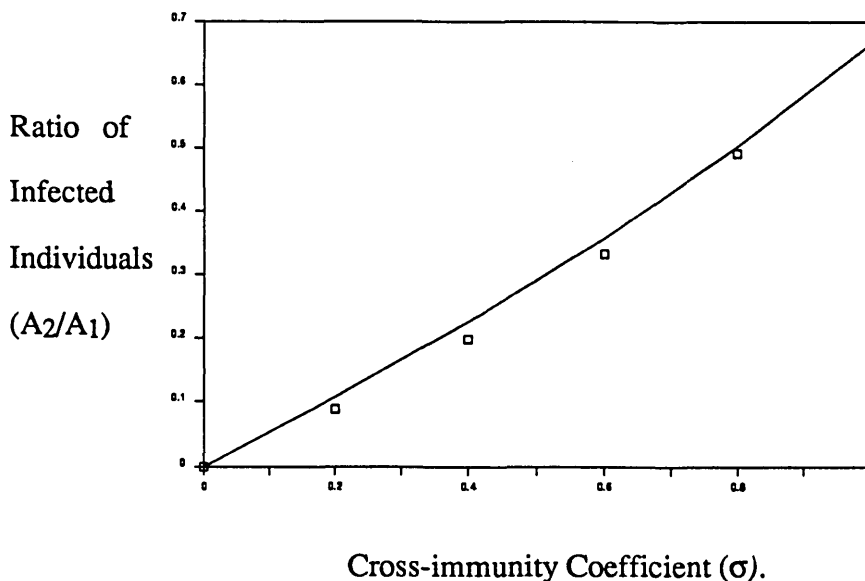
Figure 2.10; Relationship between the cross-immunity coefficient (σ) and the ratio of infected individuals (A_2/A_1), derived from numerical analysis of the differential equation system. A_1 is the number of individuals infected with one strain and A_2 is the number of individuals infected with two.

other directly transmitted viruses (see Anderson and May (1983a and 1985) for example). Therefore, it is not relevant to comment on them in this study, as they have no direct bearing on the outcome of numerical analyses unless they are greatly in excess of, or less than, the values suggested by epidemiological studies.

2.5. Discussion.

Numerical analysis of this simple non-age-structured model has given some useful insights into the dynamics of co-existing strains of the same virus. In terms of the rudimentary relationship between two strains of different infectivity which co-exist, assuming no cross immunity occurs, it can be seen that similar patterns arise to those observed from case notification data (see chapter 3). It is clear that two strains which do not interact to any great degree, or share the same pool of susceptible individuals, will cause epidemics which are varied both in their magnitude and duration depending on the values of the parameters that determine the magnitude of R_{0i}. However, it is useful to explore the possible role of cross-

immunity in the dynamics of co-existing strains, to determine what differences this effect causes on the patterns of infection within a community. From Fig. 2.9 it can be seen that the cross-immunity coefficient may well have some considerable bearing on the transmission of co-existing strains, and it has also been shown that cross-immunity causes an alteration in the behaviour of co-existing strains in terms of the observed efficiency of transmission of each strain (Fig. 2.5). Thus, by introducing an element of cross reactivity between strains, these strains are forced to compete for susceptible individuals. This very competition for susceptible individuals causes irregular patterns with respect to the reappearance of each strain of the virus (Figs. 2.6 and 2.7).

In terms of the time scale which can be imposed on the reappearance of a particular strain of a virus, it has been shown, numerically, that the same dynamics are observed between co-existing strains even if the appearance of the second strain is delayed by as much as 50 years. This indicates that, if the immunological memory is lifelong, immunity conferred on one strain of a virus by a previously circulating strain, which is antigenically similar, will also last a generation. Not until the population of susceptible individuals has sufficiently replenished itself, via new births, will the effects of cross-immunity be lost.

The two strain model consisting of ordinary differential equations, which do not take into consideration the variation of the population with age, shows that whereas variable forces of infection will alter the proportions of the population which are immune to the viruses, it is possible that the cross-immunity coefficient (in combination with these strain-specific forces of infection) causes the epidemics to take the form that is observed in case notifications (see chapter 3). The relationship between the force of infection and the size of the epidemic shows clearly that the cross-immunity coefficient is not necessary to generate the patterns that are observed from case notification data. However, it can be seen that cross immunity has a limiting effect on the size and duration of any epidemic (Fig. 2.9). If there is a significant role played by strain specific cross immunity it may well be as a limiting factor in both the size and shape of the epidemics, and also in the number of strains which can co-exist in any one season. Thus, because strains which confer immunity on each other are essentially competing for the resource pool of susceptible individuals in the community, the number of strains which can co-exist is probably limited.

The numerical analysis of the model has shown also that the effect of cross-immunity may well be long lasting, due to the time needed for the number of susceptible individuals to build up to an appropriate threshold value (via new births) after the initial epidemic. Thus, an epidemic of a new strain in a given season may well affect an antigenically similar strain in a subsequent season. This makes it imperative to examine case notification data to determine whether there are noticeable trends in the long term patterns of epidemics, or whether the epidemics appear unpredictable, which is what the basic model suggests (Fig 2.7). It is useful to consider the epidemics both in the same season, and in different seasons, to determine whether a long lasting cross immunity is observed.

The estimation of a cross-immunity coefficient is obviously problematical, since it is not possible to determine exactly which strain confers what degree of immunity against other strains in the absence of experimental infection in volunteer patients. However, an idea of the magnitude of this parameter can be obtained from case notification data, simply by comparing the size of two epidemics of different strains occurring in the same season. It must be noted that the estimation procedure is very crude since other parameters, such as the magnitude of Roi for a given strain, will influence the relative magnitude of the observed epidemic.

In terms of the reproductive rate which can be derived from the numerical analysis of the model, it has been shown that, in the same way that increased infectivity of a virus in isolation acts to raise the reproductive rate, so do the infectivities of two strains of the same virus which are co-existing. It can also be shown that cross-immunity between two co-existing strains causes the overall reproductive rate of the two strains combined, Ro', to be elevated (calculated using the single strain definition of Ro given in equation (2.7) considering the virus as a product of both strains). This is due to the pool of individuals susceptible to the virus becoming limited as a consequence of increased cross-immunity between co-existing strains of the same virus. This is of some importance, considering that the observed strain-specific reproductive rates of the strains under consideration (see Table 2.2) are higher than those predicted by the model. This might be a direct consequence of the strains being apparently epidemic each year, or it may well be the case that the observed reproductive rates are raised due to some degree of long lasting cross-immunity between the two strains.

Chapter Three: Age-Stratified Case Notifications.

3.1. Introduction.

Public health records of infectious disease notifications are a valuable source of epidemiological information in the United Kingdom. These records have been more widely used in past epidemiological studies than serological surveys (Grenfell and Anderson (1985) and Anderson and May (1983b)) especially in the study of the influenza virus (Chakraverty et al. (1982 and 1986), Pereira and Chakraverty (1977 and 1982), Glezen (1980 and 1982), Glezen et al. (1982 and 1980), Hope-Simpson (1978,1979 and 1981) and Hope-Simpson et al. (1987)). Case notifications for diseases such as mumps, measles, pertussis, rubella and influenza yield information on the change in the proportion of a community or a population who have experienced a particular viral or bacterial infection. To give suitable information on the proportional changes in the population with respect to age, the case notifications should be for a specified time interval (either a month, a year or an epidemic season) and be age-stratified (Griffiths (1974), Fine and Clarkson (1982), Grenfell and Anderson (1985), Anderson and May (1985)).

Many problems arise with the use of case notifications as indicators of infection in a population. Most important of these is the error associated with any bias in reporting which may occur. As discussed in chapter 1, the younger age groups and the elderly are more prone to developing complications from infection with the influenza virus. This will tend to result in individuals in these age groups seeking consultations with their General Practitioners far more frequently than those persons in age groups which are less likely to develop complications. Thus more positive 'cases' will occur in the very young or elderly age groups. The second problem is that involving the distinction between infection and disease. To successfully obtain data for the estimation of the epidemiological parameters which are central to the transmission dynamics of directly transmitted viruses it is essential that the proportion of the population **infected** with the virus is known. Case notification data, however, will be biased towards those individuals who show clinical signs of disease, since these individuals will be more likely to be in a situation involving the routine sampling of blood. It is these

routine blood samples which are used in the diagnosis of viral infection from which the notification data is acquired (which in itself is limited by the accuracy of the test used to detect the specific strain of influenza). Therefore, this data will be biased towards cases of disease, as opposed to cases of infection. Taking all this into account, however, it is still possible to assume that the results will maintain a constant bias through time, and thus be comparable between epidemic periods. It should also be possible to compare these findings with those obtained through the analysis of serological data.

Age related trends in influenza A case notifications (obtained by the Public Health Laboratory Service (PHLS) from various sources in England) are explored in this chapter with the following three aims;

(a) To determine if there are consistencies in important characteristics such as the age-related nature of influenza A transmission rates between the two sources of information, and if there are not, to explain why. It is also useful as an additional source of information where serological data is lacking. It can thus provide epidemiological information of a different sort from that derived from serological surveillance as presented in chapter 5.

(b) To determine if there are any trends in the occurrence and size of influenza A epidemics, both in terms of subtypes and strains, other than the obvious seasonal variation, and to explore any temporal trends in the average age at infection. This is studied by the analysis of the incidence of the influenza A virus (detailed in section 3.5.).

(c) To justify the need for influenza epidemiological data based on serology by establishing that not only is there a paucity of case notification data, but also that there are serious limitations in the assumptions underlying the analysis of case notifications (see section 3.3).

3.2. Sources of Case Notifications.

Influenza is not nationally notifiable in the UK in contrast to, for example, measles, whooping cough and diphtheria. However numbers of confirmed influenza infections discovered in routinely collected blood samples by Public Health laboratories (referred from General Practices and hospitals) are collated by the Communicable Disease Surveillance Centre (CDSC), in Colindale, London.

Unfortunately, data that are divided by age for specific strains are only available for one epidemic season (from Chakraverty,P. - pers. comm.), namely that of 1982/83. In addition, the data presented is grouped into inconvenient age classes (the raw data was not available).

The temporal trends in the occurrence of epidemics from 1973 to 1989 is presented in Fig. 3.1 (data from the PHLS weekly reports), with the data age stratified and subtype-specific for the years 1977 to 1985 in Fig. 3.2 (from Chakraverty (1982) and Periera and Chakraverty (1977 and 1982)). Unfortunately there is no reliable data available from the CDSC for weekly case reports for any years before 1973, nor are the subtype-specific data from 1985 onwards published. In most yearly seasons there are other strains present which do not constitute an epidemic, as well as there being some cases of influenza B. However, those strains which do not cause sufficient cases to enable them to be considered as causing an epidemic (see chapter 1 for details) are assumed to have a negligible effect on the immune status of the population, and are thus not analysed in conjunction with those strains which do cause excess mortality. Details of these sub-epidemic strains can be found in the papers by Chakraverty (1982) and Periera and Chakraverty (1977 and 1982).

Number of

Reports.
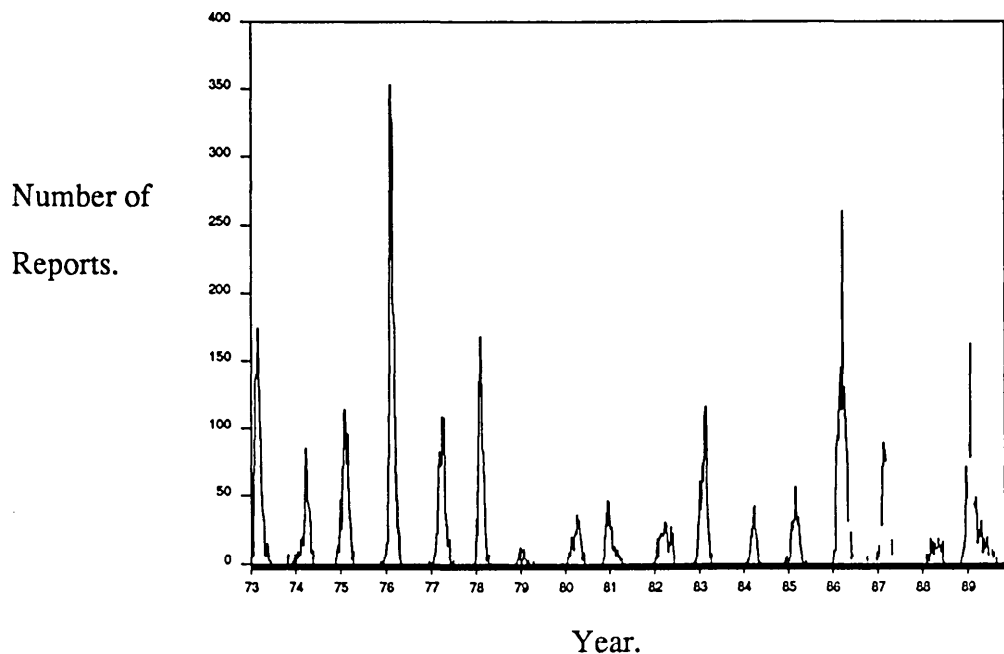
Year.

Figure 3.1; The annual epidemics of influenza A from 1973 to 1989. The seasonal trends in epidemics is clearly shown, with the epidemics occurring approximately at the beginning of each year (in months January to May). Data from the weekly PHLS reports.

400

Number of
Reports.

77   78   79   80   81   82   83   84   85
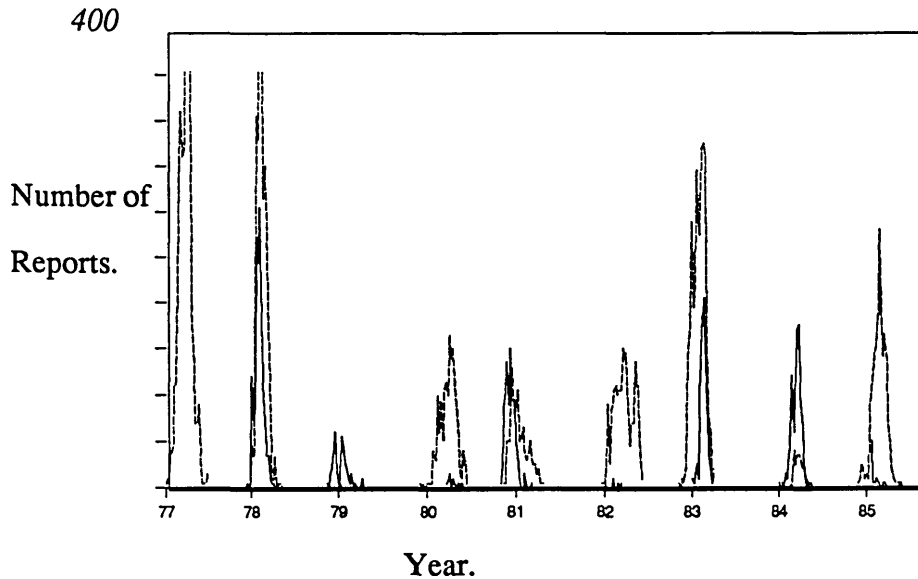
Year.

*Figure 3.2; The reported cases of influenza A from 1978 to 1985, showing the subtypes and
strains of the virus. The dotted line represents the H1N1 subtype, and the solid line rep-
resents the H3N2 subtype, new strains occur each year. Data from Chakraverty et al.
(1982, 1986) and Perriera and Chakraverty (1977).*

It can be seen from Fig. 3.2 that influenza A causes yearly epidemics, with the major
peak in incidence in the first 14 weeks of a new year. It can also be seen that in most years
two subtypes are present, with one subtype always causing a larger, earlier epidemic than
the second strain.

A further analysis was conducted on the data, to determine whether there was any corre-
lation between the epidemics of each subtype. Cross correlations were carried out between
the two time series for both seasonally adjusted, and seasonally unadjusted data, with the as-
sumptions that the series of the H1N1 subtype was leading that of the H3N2 subtype and
vice versa (Chatfield (1975)). Those results which showed a significant correlation between
the two subtypes are; both sets of data with seasonal trends removed and the normal data for
the H3N2 subtype followed by the H1N1 subtype. These results are shown in Figs. 3.3 and
3.4. It can be seen that the most significant correlation is that for H3N2 leading H1N1, where

a)

Correlation

Coefficient.

Lag (k)

b)

Correlation

Coefficient.

Lag (k)
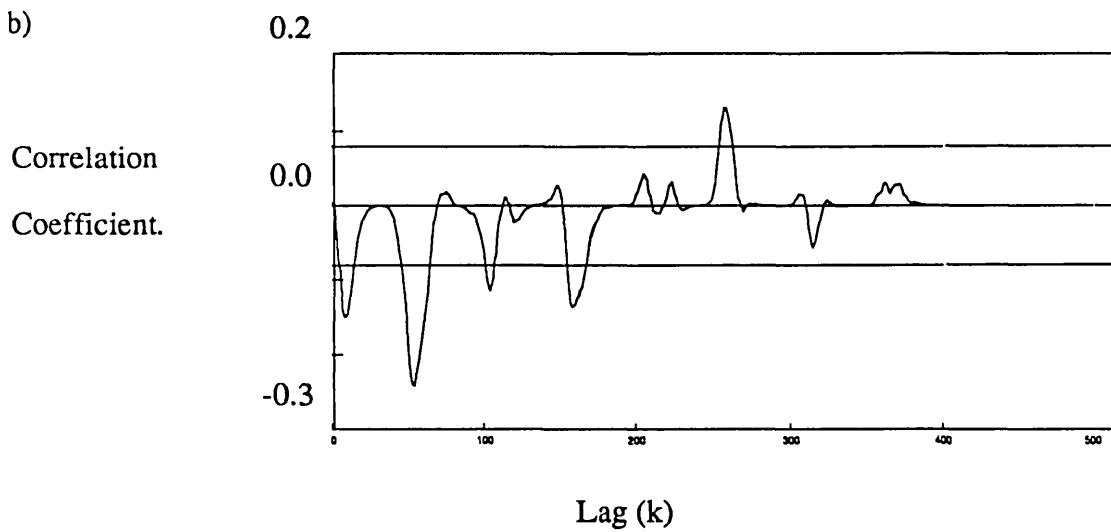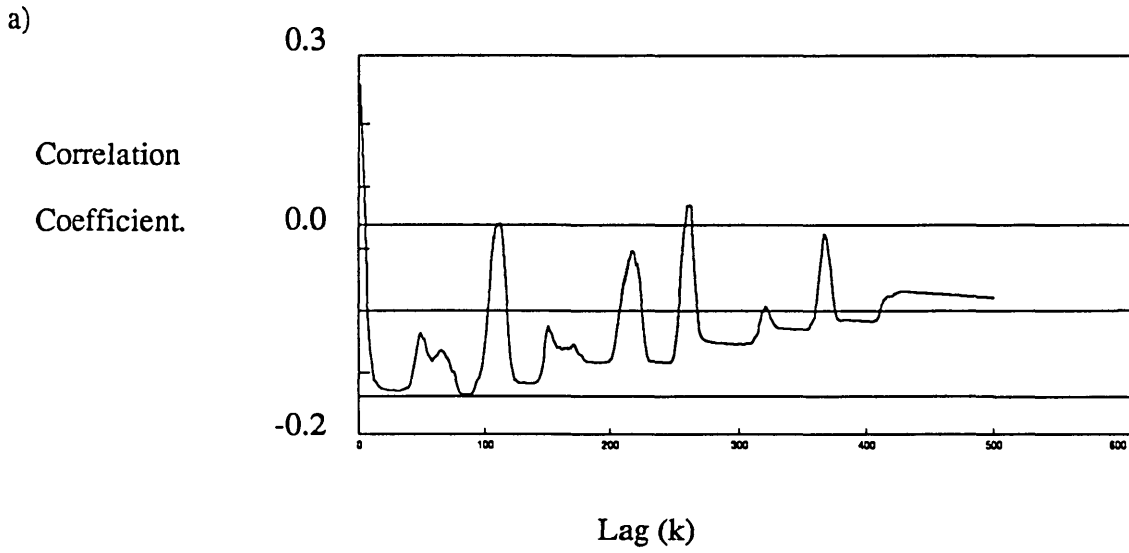
Figure 3.3; The cross correlation results for the case where the H1N1 subtype leads the H3N2 subtype, (a) with no correction for seasonality and (b) seasonally corrected. The top and bottom horizontal lines represent the 95% confidence limits. The centre line denotes zero correlation.

a)
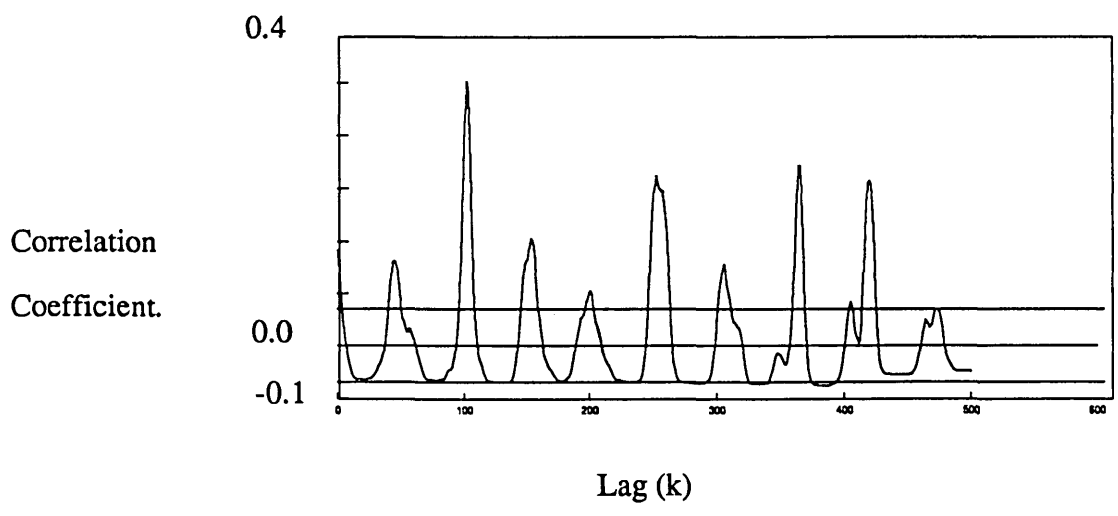
b)
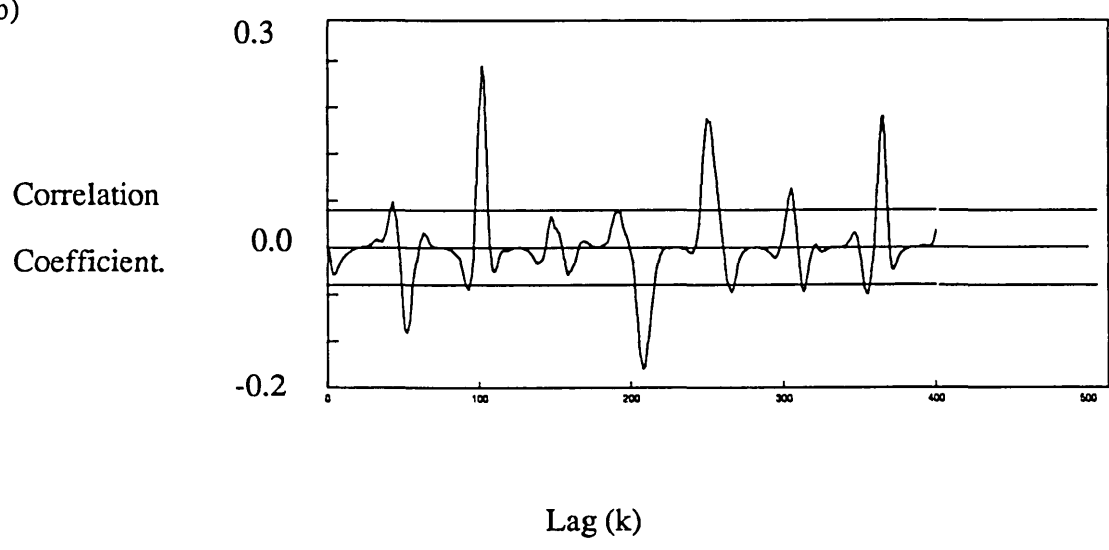
Figure 3.4; The cross correlation results for the case where the H3N2 subtype leads the H1N1 subtype, (a) with no correction for seasonality and (b) seasonally corrected. The horizontal lines are as defined in Fig. 3.3.

150

Number of

Reports.

0

26 28 30 32 34 36 38 40 42 44 46 48 50 52 2 4 6 8 10 12 14 16 18 20 22 24

Year.
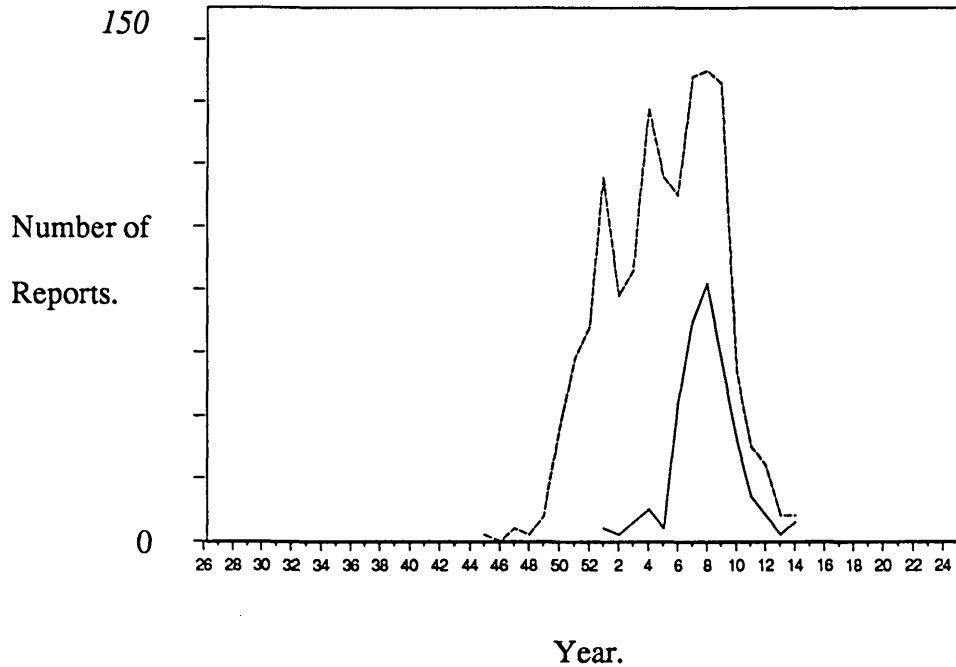
Figure 3.5; The reported cases of influenza for the 1982 to 1983 period, showing the two major strains involved. The dotted line represents A/Eng/333/80 (H1N1) and the solid line represents A/Bel/1/81 (H3N2). Data from Chakraverty et al. (1986).

there is a good positive correlation (Fig 3.4(a)). This implies that (due to the effect of seasonality) if H3N2 occurs in the population before H1N1, it is very likely to be followed by an epidemic of H1N1 in the same year. However, no other obvious patterns are clear.

Figure 3.5 shows in more detail the epidemic of the season 1982/83, with the two subtypes in evidence. In this case the two strains (each a different subtype) were A/Bel/1/81 (H3N2) and A/Eng/333/80 (H1N1), which are discussed throughout this chapter and are considered with respect to age specific serology in chapter 5. The point to observe from Fig. 3.5 is the basic shape and pattern of the epidemics; this is of relevance to the numerical analyses reported in chapter 2. This pattern is consistent with that described by Glezen et al. (1982) as being a 'Herald Wave'. Glezen considered that the smaller of the two epidemic curves was indicative of the presence of the next strain of virus that will cause an epidemic

in the following season. However, after several years no further Herald Waves were observed, which essentially disproved this theory. It is still useful to note that the consistent shapes and recurrence of the 'bimodal' epidemic curves are observed both in Texas (Glezen et al (1980a, 1980b, 1982a and 1982b)) and in England and Wales (Chakraverty et al. (1982 and 1986)). It is this bimodal shape of epidemic that is a characteristic feature of co-existing influenza strains. In this thesis the concept that this pattern could represent the result of cross-immunity between two co-existing virus strains is considered. It is proposed that these patterns are observed due to individuals becoming infected with the first strain of virus, which reduces the size of the susceptible population for the next strain. Therefore, subsequent epidemics of either the same virus, or antigenically similar viruses, will be both smaller, and temporally more separate. This follows on from the results generated by the numerical analysis of the basic mathematical model described in the preceding chapter.

Part I. Age-dependent Variation in Transmission.

3.3. Methods of Analysis.

For the purposes of investigations of this type it is assumed that the accumulated proportion of cases, with age, from notifications reported over one season mirrors the cumulative infection of a cohort of individuals from birth through time (Anderson and May (1985)). Recovery from many acute viral infections confers lasting immunity to re-infection, and as a consequence these data are taken to represent age-related changes in the proportion of the population who have specific immunity.

Implicit in this assumption is that the rate of transmission for the infectious disease agent is constant through time. Seasonal fluctuations in rates of infection are well documented (for example Fine and Clarkson (1982)), and infection rates also vary over epidemic cycles and even longer periods (Grenfell and Anderson (1985)). In these studies the supposition that horizontal age-stratified information reflects longitudinal trends applies equally well to case reports as it does for serological data (Anderson and May (1982b and 1985), Grenfell and Anderson (1985)). However, in this case, these assumptions do not hold true. Because the virus strains are epidemic over single seasons, and do not reach an endemic equilibrium, the assumption that the accumulated proportion of cases with age mirrors the cumulative infection of a cohort of individuals from birth through time is incorrect. Since there is an obvious marked change in the rate of transmission through time (specifically from zero to some positive value over the course of the first epidemic) this method cannot be used to measure the force of infection. In fact, due to the epidemic nature of the influenza virus, no precise value can be attributed to the age-specific forces of infection for each strain. However, some idea of the age-dependent nature of the transmission of the strains can be obtained by simple observations of the case notification patterns with respect to age.

For case notifications the assumption must be made that every age group is reported with equal efficiency (Collins (1929), Fine and Clarkson (1982)), i.e. there is no age related bias in reporting. It is very difficult to determine to what extent over or under reporting occurs

in influenza cases, but to help alleviate this problem, all data is weighted by the proportion of the population that the age group in question occupies in the population as a whole.

The case notification data is considered at two levels. Initially it is assumed that there is no strain or subtype interference. In other words only the influenza A virus is taken to be in circulation. Secondly the subtypes (which are strain classified where possible) are treated as being independent of each other, thus giving a crude reflection of the impact of cross-immunity between the two strains, if any.

3.3.1. Average Age of Infection.

It is useful to examine the average age at which individuals become infected, since this gives an indication of the force of infection of a given strain in a community with co-existing strains of a virus. Thus, if the average age of infection is low the net force of infection is high, and vice versa. It must be emphasised that this average age is not analogous to the average age at first infection with a virus which is known to be endemic in a community, since there can be no guarantee that there is a constant force of infection acting on each age group over the years studied. The concept of endemic versus epidemic virus strains is dealt with in detail in section 5.7.3. However, the weighted mean age of infection does give an indication of the variability of the forces of infection (which are equivalent to the reproductive potentials) of the different strains of the same virus.

The average age at infection can be calculated by determining the weighted mean age of infection from the case notification data for each year. Two values are given here for the average age, due to the considerable difference that is observed due to the inclusion or exclusion of the 65+ year age group. Since it is suspected that this age group may be affected by a considerable bias from over reporting, it is considered that the real average age lies somewhere between the two. Table 3.1. shows the average ages of infection for the two subtypes over the years 1979 to 1989, and compares them to estimates made from other studies. By comparison with the average ages of infection from the serological study (Fig. 5.8), it can be seen that there is considerable agreement between the values.

Figure 3.8 shows the change in the average age at infection over the time period studied. From this it can be seen that there is not a great deal of variation between the ages, although these values may be a result of different strains being introduced into the population at each

season. Unfortunately, the data available does not cover a long enough time period to perform any detailed analysis on the changes in average age at infection through time, or to determine whether there are any long term patterns. The average age of infection is normally a useful indicator of transmission rates in that it is indirectly proportional to the forces of infection acting on individuals in the younger age groups; if the average age is high it indicates that the forces of infection were low in the young and vice versa (see Table 3.1).

Table 3.1;Estimates of the Average Ages of infection from case notification data. Estimates were made using the method described in the text, with estimates in brackets indicating that the 65+ age group is not included in the calculations.

Strain/Subtype	Year	Age	Source
H2N2	1957	34.6	Hennessy *et al.* (1964).
H2N2	1960	30.0	Hennessy *et al.* (1964).
H3N2	1966-1971	21.4	Monto and Kioumehr (1975).
A/HK/68	1968	28.7	Zachary and Johnson (1969).
H3N2	1974-76	18.2	Glezen and Couch (1978).
H3N2	1977-78	21.9	Glezen *et al.* (1982).
H1N1	1978-79	12.9	Glezen *et al.* (1982).
A/Tex/1/77	1979/80	41.2 (16.5)	PHLS weekly reports.
A/Bangkok/1/79	1980/81	32.9 (13.9)	PHLS weekly reports.
A/Eng/333/80	1981/82	32.2 (13.8)	PHLS weekly reports.
A/Bel/1/81	1982/83	34.3 (15.3)	PHLS weekly reports.
A/Phil/82	1983/84	25.4 (17.5)	PHLS weekly reports.
A/Phil/82	1984/85	28.2 (13.7)	PHLS weekly reports.
A/Chile/83	1985/86	36.8 (18.5)	PHLS weekly reports.
H3N2	1986/87	32.3 (21.2)	PHLS weekly reports.
H3N2	1987/88	29.9 (19.4)	PHLS weekly reports.
H1N1	1988/89	36.6 (14.6)	PHLS weekly reports.
A/Eng/333/80	1982/83	8.2 (23.4)	Chakraverty (pers. comm.)
A/Bel/1/81	1982/83	11.6 (14.1)	Chakraverty (pers. comm.)
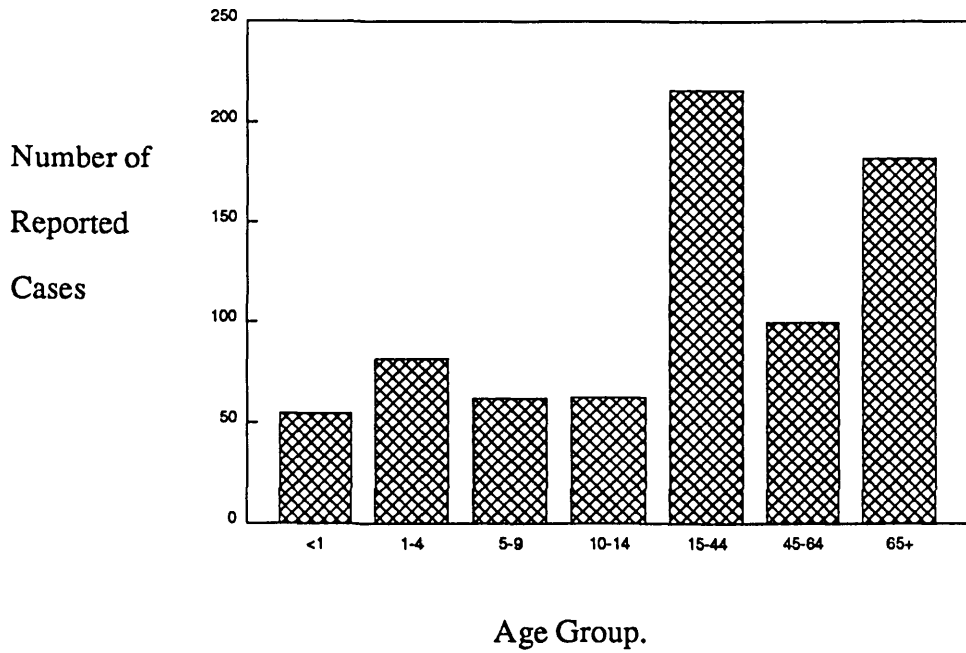
Figure 3.6; The average number of case notifications for all influenza strains with respect to age from 1979 to 1989. Data from the PHLS weekly reports. Note that the case reports do not exceed 250 per age class.
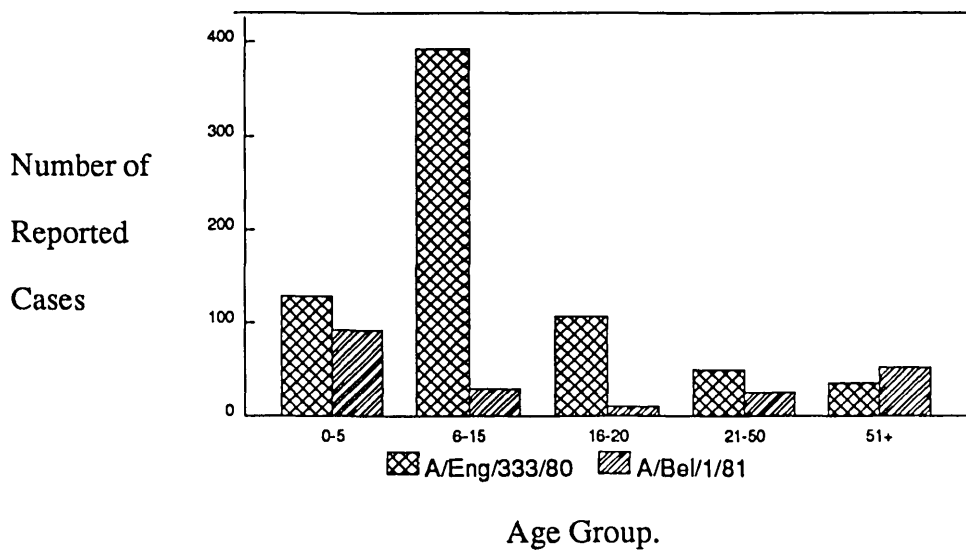
Figure 3.7; The age-specific number of case notifications for A/Bel/1/81 and A/Eng/333/80 respectively, for the 1982/83 season. Data is from Chakraverty (pers. comm). Compare the maximum number of reports per age group with that of Fig. 3.6.

Age Group.

Figure 3.8; The average ages of infection with influenza A for the time period 1979-1989, estimated from the data shown in Fig 3.2. For details of method of estimation see text. The left bar represents the average age with the 65+ age group included, the right bar is the average age estimated excluding this age group

The average ages of infection for the strains which were co-existing during the season 1982/83 (A/Eng/333/80 and A/Bel/1/81) are dissimilar to that observed for influenza A regardless of strain (somewhere between 20 and 30 years old); both values are much lower than those found for influenza A regardless of strain, although this may be an artefact of the age grouping (the data obtained for the strain-specific analysis was already grouped into different ages to that from the CDSC). It can be seen that the average age for infection with A/Bel/1/81 is higher than that for infection with A/Eng/333/80 (between 8 to 23 years and 11 to 14 years respectively). The H1N1 subtype, which includes the strain A/Eng/333/80, has been circulating for a longer time than the H3N2 subtype (A/Bel/1/81) which may explain the higher average age at first infection; more immunity has been built up against the H1N1 subtype in the older age groups. Variation in the average ages of infection can imply one of a number of things. For example, the virus could have a differential rate of infection for the older age groups, and perhaps infect older individuals at a slower rate than young individuals. It could also imply that, in the same way that immunisation programmes raise the average age of infection, cross-immunity between two similar strains acts to raise the aver-

age age over that which would be observed if only a single virus strain was circulating. This latter explanation appears likely if cross immunity has a significant effect.

It would be useful to compare the average ages of infection over the time periods for which each strain appears to dominate the other; namely, 1973-78 for the H3N2 subtype and 1978-85 for the H1N1 subtype (and also presumably pre-1968, before the H3N2 subtype began to circulate). If cross immunity is important, the average age should be higher for the period during which the H1N1 subtype reappeared (when other strains were circulating) than during the period in which it first appeared. Unfortunately the data needed to determine this is not available.

3.3.2. Age-dependant patterns of Infection.

From Fig. 3.6 it can be seen that most of the observed cases occur in the 15-44 year age group and the 65+ year age group. If the possibility of over reporting in the 65+ age group is considered, then it seems probable that the highest forces of infection are being observed in the 15-44 year age group. This is also in agreement with the average ages of infection of between 20 and 30 years old which were recorded in the earlier section (Table 3.1). Further stratification of this age group is desirable to determine which age classes within this group are responsible for these age effects. Unfortunately, the data (supplied by PHLS weekly reports) does not allow finer analyses. It would be useful to determine whether the observed high numbers of infected individuals in this age group are in fact of school age, which would support the findings of Glezen (1982) and Fine and Clarkson (1982), who surmise that the observed patterns of infection are due to intensive mixing in secondary schools.

It can be seen from Fig. 3.7 that there is considerable variation between strains in terms of the age-specific patterns of infection. Not only do the two strains differ in the pattern of the age-specific case reports, but also in the magnitude of these reports. It is also very noticeable that the two strains shown specifically for the 1982/83 season are by no means representative of the average pattern for all years (Fig. 3.6). Therefore, the results from the 1982/83 season must be considered with considerable caution. However, it is apparent that there is considerable disparity between the infection patterns of the different strains of the same virus with respect to age.

It is important to note that the epidemiological investigation of case reports does not provide a sound data base for estimating average ages at infection, or age-specific patterns of infection. The very identification of the strain type identified in these reports relies on the serology of the samples, which are initially intended for other purposes, and are thus not as specific as the serological tests performed in the following chapters.

Part II. Temporal Trends in the Incidence of Influenza A.

3.4. Predicted epidemic cycles for endemic influenza.

In a stable population, any directly transmitted virus that induces life-long immunity and which is at an endemic equilibrium will tend to exhibit oscillations in the densities of the susceptible and immune populations that are unrelated to seasonal factors (Anderson and May (1978a)). Non-seasonal oscillations which in the epidemic behaviour of influenza will be due to the decay and renewal of the susceptible population. Decay is caused by infection and subsequent recovery to the immune status, while renewal is due to the recruitment of new-born individuals. Simple deterministic models, such as the one presented in Chapter 2, predict damped oscillations with an inter-epidemic period, T, approximately given by (Anderson, Grenfell and May (1984))

$$T = 2\pi(AK)^{1/2} \tag{3.2}$$

where,

A = the average age at first infection

K = the average interval between an individual acquiring infection and passing it on to a new infective (approximately the sum of the latent plus infectious periods (Anderson and May (1982b)).

Table 3.2. Inter-epidemic periods for Measles, Mumps, Pertussis and influenza in the absence of vaccination strategies

Infection	Duration (K)	Average Age	Inter-epidemic Period (yrs).	
	(Days)	(Years)	Predicted	Observed
Measles[*]	12.0	4-5	2.25-2.50	2
Mumps[*]	19.0	6-7	3.50-3.80	3
Pertussis[*]	25.0	4-5	3.30-3.60	3
Influenza[+]	3.0-6.0	5.5-7	2.4	1

[*] Data from Anderson And May (1984).

[+] Estimated from serology (described in Chapter 5).

Table 3.2 shows the predicted and observed inter-epidemic periods for measles, pertussis and mumps before the introduction of mass immunisation (from Anderson, Grenfell and May (1985)) compared to that for influenza. The durations of infection and the estimated average ages at first infection are shown, from which the inter-epidemic period was calculated. It must be emphasised that the average age at infection, as calculated earlier, does not specifically reflect the time taken, after birth, to become infected with the virus, but is in fact the weighted mean of the age at which individuals become infected during an epidemic. However, taking this into account, and with no other method for determining the average age at infection, these values can still be used to make an estimate of the predicted inter-epidemic period. Using these values, it can be seen that an influenza strain is likely to show non-seasonal epidemics every 50 to 75 years (depending on which age at infection is used). These values are much higher than those observed for the classical childhood diseases. Obviously, the data used here does not span a large enough time period to test this hypothesis, however, it is useful to note that this period agrees with that predicted by the mathematical model presented in the earlier chapter.

By attempting to determine what the real inter-epidemic period is, it should be possible to make some assumptions about the endemicity, or otherwise , of the influenza virus. If the virus is at endemic equilibrium, non-seasonal patterns should emerge. If, however, the epidemics are being driven by seasonal factors (mixing behaviour dictated by weather conditions for example) and by the chance arrival of new strains such patterns will be less apparent in observed trends.

3.5. Time Series Analysis.

In this investigation time series analysis is used to study temporal patterns in both the number of reported cases of infection, and in the variation in the average age at infection for the epidemics to determine whether any long-term oscillations which might occur are those predicted by the theory of waxing and waning of immunity in the population. The time period covered in both cases is that which is considered in the serological studies (i.e.1973 to 1989) since no accurate strain-specific case-notification data exists for the four-year period 1969 to 1972.
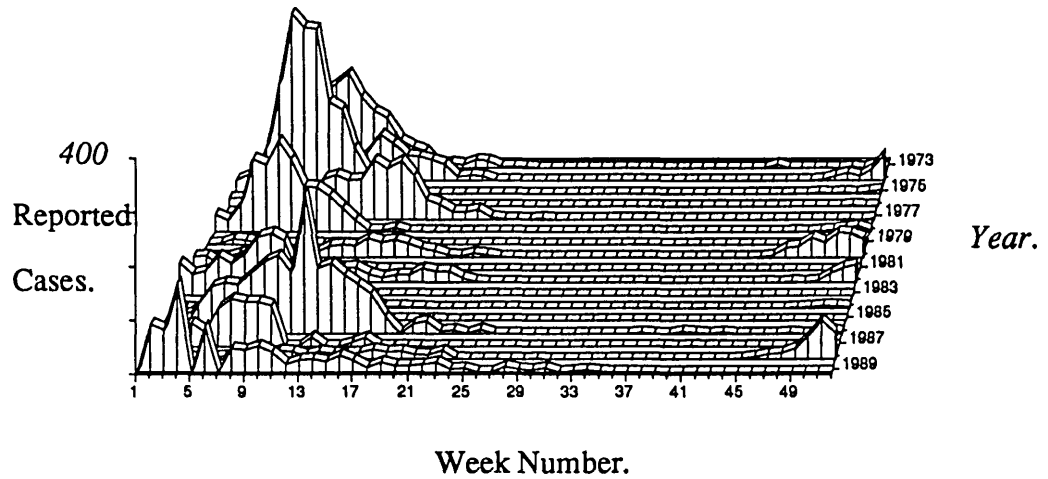
Figure 3.9; The annual epidemics of influenza A from 1973 to 1989, shown by week number, where week 1 is the first week in January. The seasonal trend of yearly epidemics is clearly observed. Data is from the weekly PHLS reports.

The annual pattern of epidemics for this time period can clearly be seen in Fig. 3.9. This figure does not break down the data into the different strains, or subtypes, but simply shows the trends in influenza A as a whole.

Two complementary techniques are employed here for examining the periodicities in the observed time series; autocorrelation and spectral analysis. A more detailed description of the statistical methods employed can be found in Jenkins and Watts (1968) and Chatfield (1975), and only the a brief summary is presented in the following subsection.

3.5.1. Autocorrelation.

This technique is based on the determination of a series of coefficients which reflect the correlation between data values (e.g. number of cases or average age at infection) at different times during the period under study. In particular it is useful to measure the correlation between the original data and the same data series after it has been displaced through time by a specific amount (the time lag k). The value of a particular correlation is represented by the symbol r_k which denotes the correlation with a time lag of k time units. Autocorrelations are usually interpreted using a correlogram, which is simply a plot of the correlation between

a)

Lag

Coefficient

(r_k).

Lag (k)

b)

Spectral

Density

Frequency $(year^{-1})$
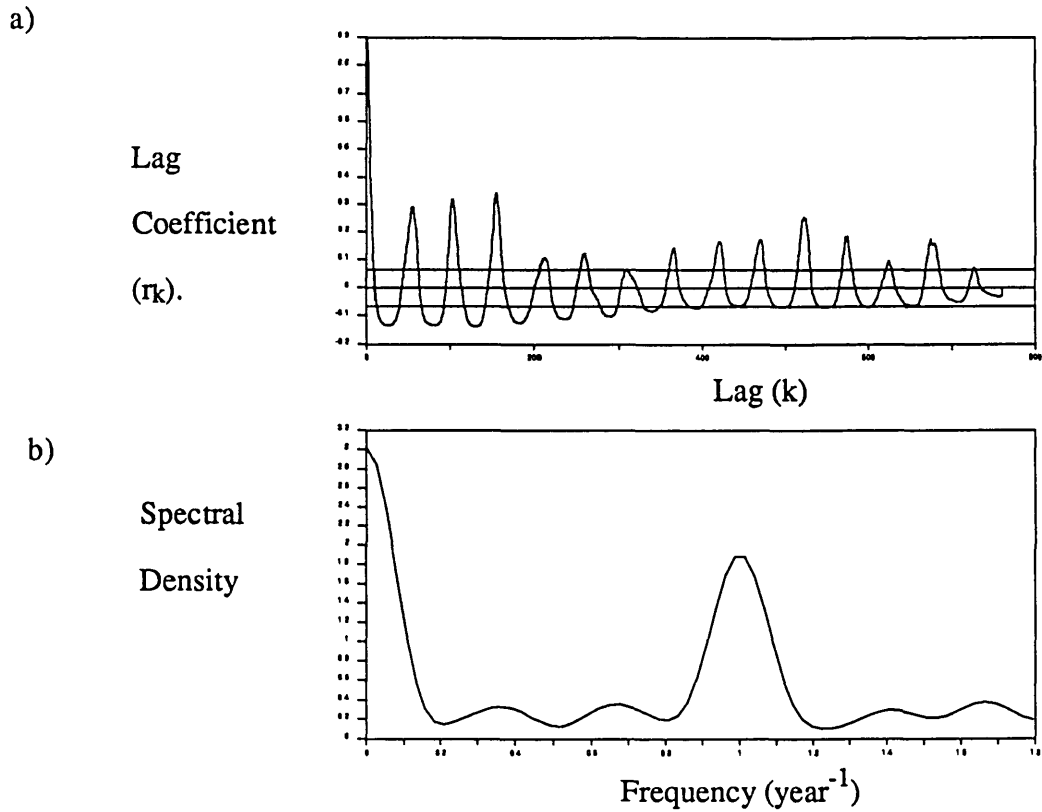
Figure 3.10; a) Correlogram and b) spectra for the case notification data for the period 1973-89 (shown in figure 3.1) for influenza A, where r_k is the lag coefficient for the lag k. The horizontal lines represent the 95% confidence limits. See text for details.

c)

Lag

Coefficient

(r_k).

Lag (k)

d)

Spectral

Density

Frequency $(year^{-1})$
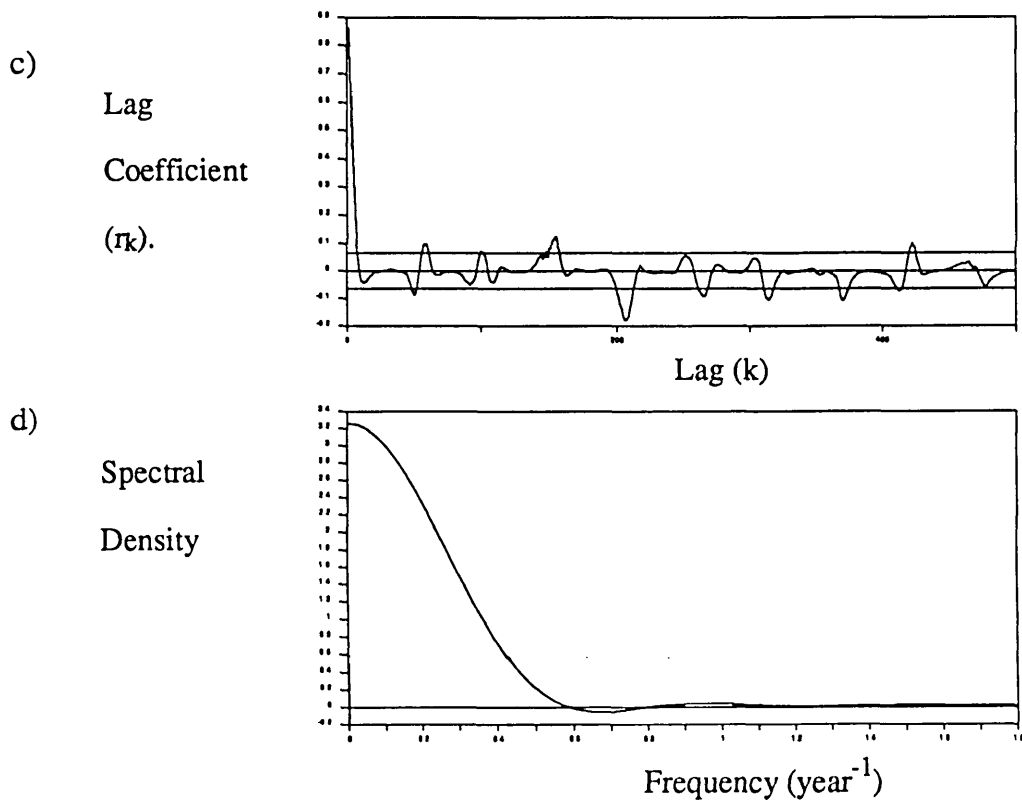
Figure 3.11; a) Correlogram and b) spectra for the seasonally corrected case notification data for the period 1973-89, for influenza A, where r_k is the lag coefficient for the lag k. The horizontal lines represent the 95% confidence limits. See text for details.

the original data and the lagged data (r_k), against the lag itself (k). It is then possible to determine confidence limits by considering a correlogram based on randomly distributed data and to test for a departure from randomness in the test series (Anderson, Grenfell and May (1984)).

Regular fluctuations in a time series will tend to generate oscillations at the same frequency in the correlogram.

3.5.2. Spectral Analysis.

In contrast to the correlogram, which is a useful tool for analysing observed periodicities in time, spectral analysis considers all possible oscillations at different frequencies, making it possible to determine whether any are relevant to the series under study. The values of the spectra represent the difference between the total variance of the series and components of

a)

b)
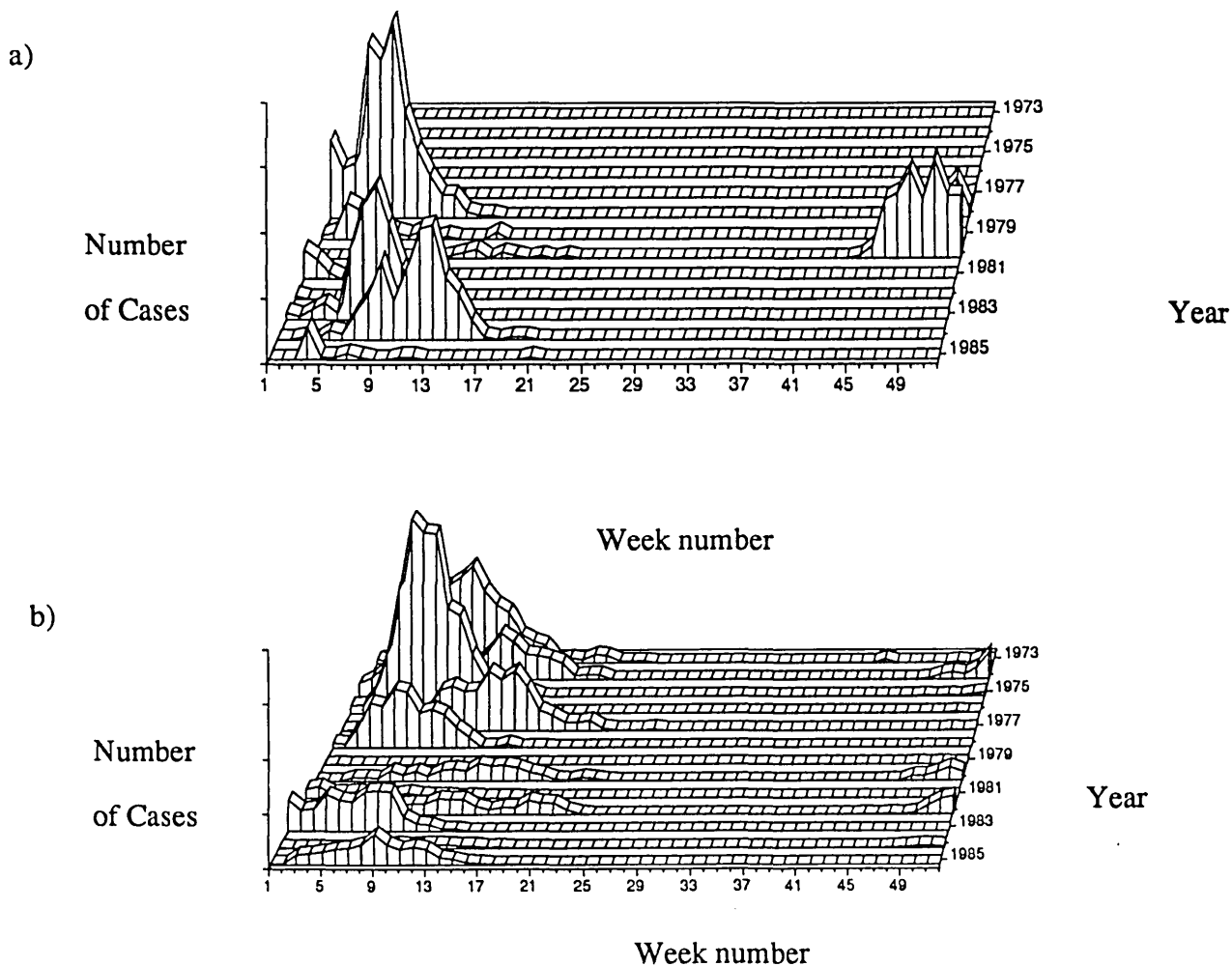
Figure 3.12; The subtype-specific annual epidemics of influenza A, shown by week number, where week 1 is the first week in January; (a) A/Eng/333/80 (H1N1), (b) A/Bel/1/81 (H3N2). The seasonality in epidemics is evident. Data is from Periera and Chakraverty (1982) and Chakraverty et al. (1986).
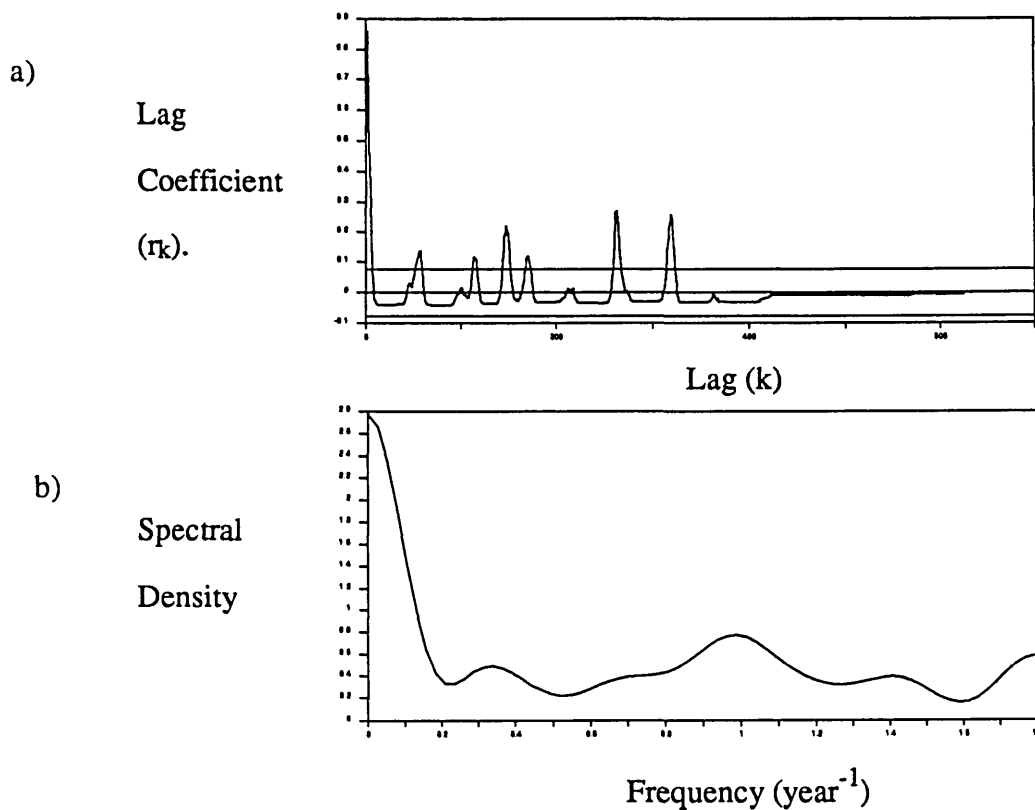
Figure 3.13; a) Correlogram and b) spectra for the strain specific case notification data (shown in figure 3.12(a)) for the H1N1 subtype of the period 1979-85, where r_k is the lag coefficient for the lag k. The horizontal lines represent the 95% confidence limits. See text for details.
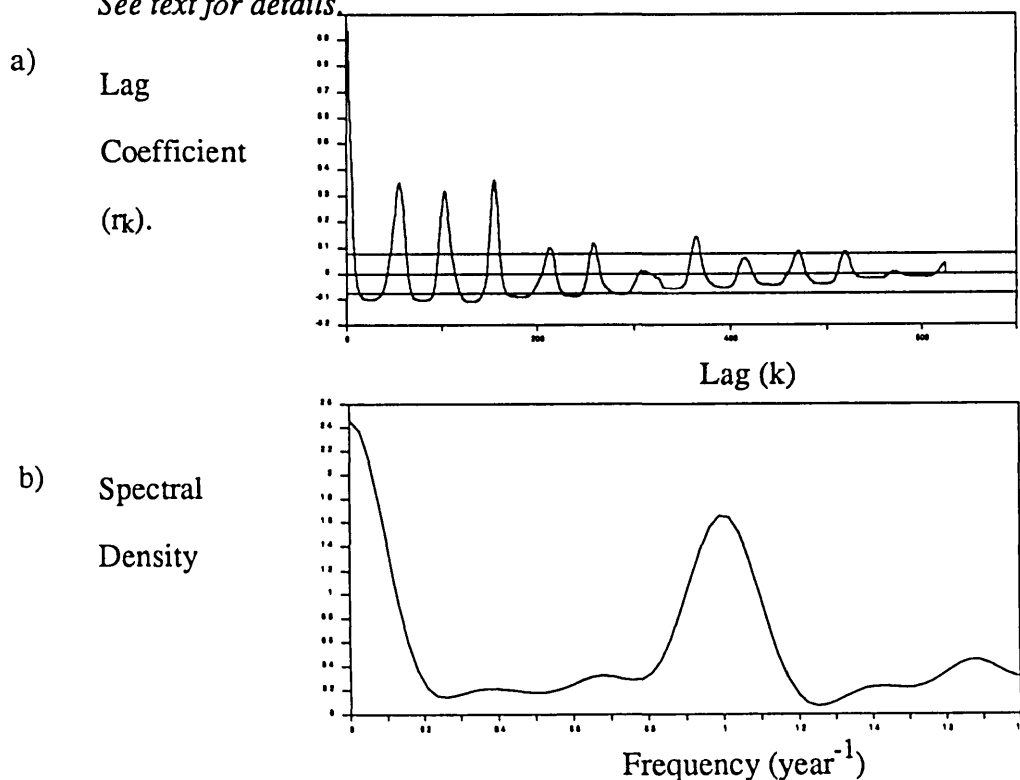
Figure 3.14; a) Correlogram and b) spectra for the strain specific case notification data (shown in figure 3.12(b)) for the H3N2 subtype of the period 1979-1985, where r_k is the lag coefficient for the lag k. The horizontal lines represent the 95% confidence limits. See text for details.

a)

Lag

Coefficient

(rk).

Lag (k)

b)

Spectral

Density

Frequency (year^{-1})
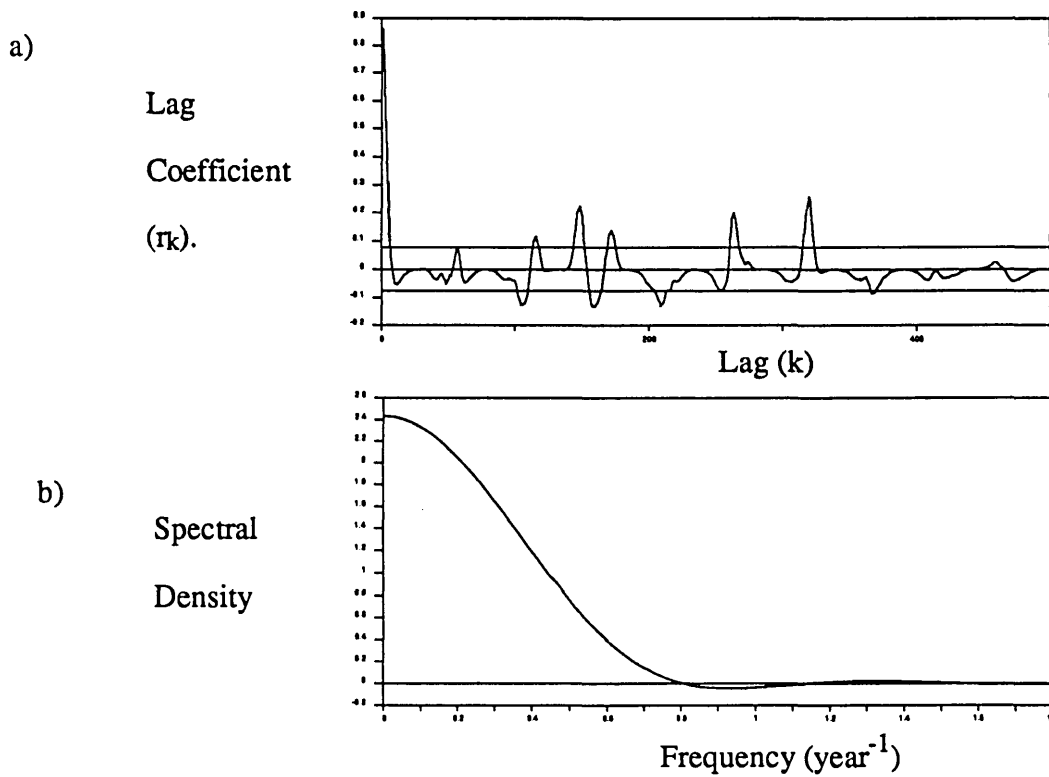
Figure 3.15; a) Correlogram and b) spectra for the strain specific seasonally adjusted case notification data (shown in figure 3.12(b)) for the H1N1 subtype of the period 1979-1985, where r$_k$ is the lag coefficient for the lag k. The horizontal lines represent the 95% confidence limits. See text for details.

a)

Lag

Coefficient

(rk).

Lag (k)

b)

Spectral

Density

Frequency (year^{-1})
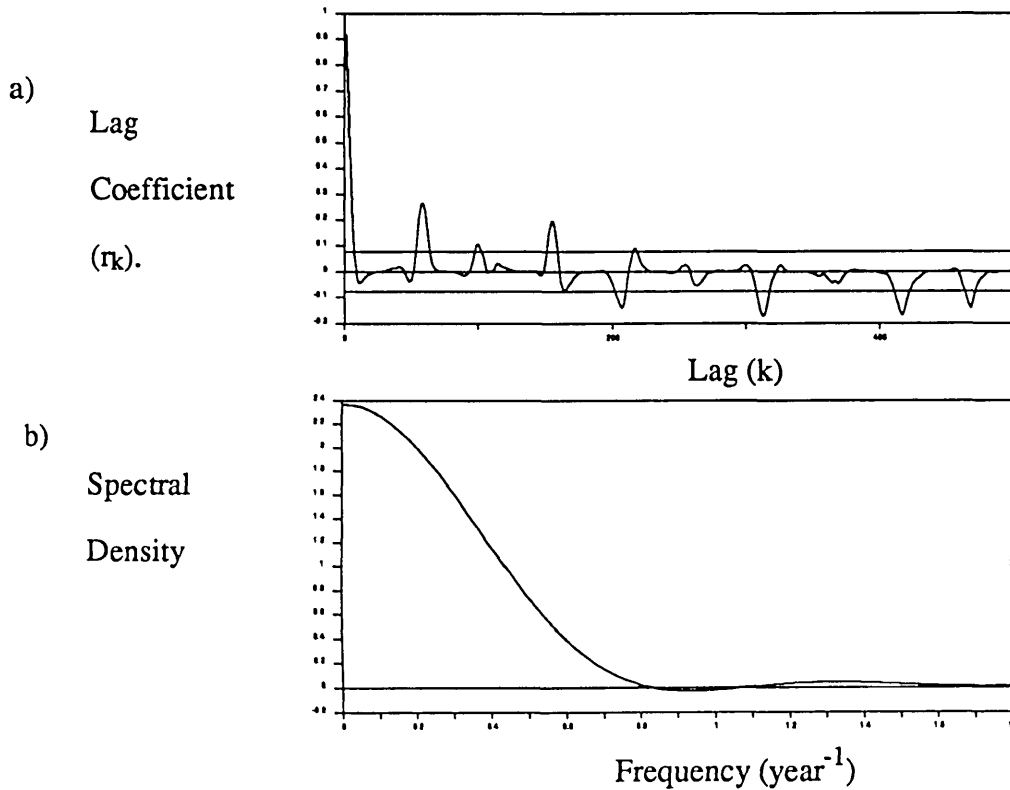
Figure 3.16; a) Correlogram and b) spectra for the strain specific seasonally adjusted case notification data (shown in figure 3.12(b)) for the H3N2 subtype of the period 1979-1985, where r$_k$ is the lag coefficient for the lag k. The horizontal lines represent the 95% confidence limits. See text for details.

a sinusoidal oscillation. Therefore the maximum value the spectra can represent is a frequency of one cycle for every two observations (which is directly measurable). Varying degrees of spectral smoothing are used, represented by the number of cycles per year, which give different values for the 95% confidence limits. Since the data is log transformed to reduce the asymmetry of the epidemic peaks, and the results are plotted unlogged, these confidence limits are multiplicative.

Regular oscillations in a time series will produce a spectrum with a sharp peak at the frequency of the oscillation, with smaller peaks at integer multiples of this frequency.

3.6. Observed Periodicity of the Influenza A Virus.

As with the force of infection estimates, the data is treated first as influenza A reports irrespective of strain, and second with subtype specific considerations, assuming the strains to be influenced by cross-immunity.

3.6.1. Influenza A Infection without Subtype or Strain Classification.

The correlogram of influenza A case notifications, a graph of the serial autocorrelation coefficients plotted against the time lag in years, is shown in Fig. 3.10(a). The smoothness of the correlogram indicates a considerable regularity in the seasonal 1-year cycles. It can also be seen that the seasonal pattern of annual epidemics (denoted by a frequency of 1.0 per year in the spectra shown in Fig. 3.10(b)) is sustained for different values of the lag k.

If, however, the seasonality is removed by subtracting the mean weekly reading, taken from the whole data set of notifications, from each annual weekly reading, no periodicity is observed. This is shown in Fig. 3.11(a), where it can be seen that no regular period is recurrent above the 95% confidence limits. This is confirmed by the spectral analysis (Fig. 3.11(b)) from which no peaks can be ascertained, for any frequency of oscillation.

There does not appear to be any other component causing oscillations in the epidemics of influenza A other than this annual seasonal variation. Non-seasonal periodicity, however, is clearly exhibited by measles, pertussis and mumps (Table 3.2). This indicates that the influenza virus is introduced into a population of susceptibles each new season, and does not, therefore, behave like other endemic viruses, with the 2.5 year non-seasonal epidemic cycles which are predicted by the theory described in section 3.5. It is possible that with a longer-

term data set some patterns may emerge, but it appears more likely that new strains arise as essentially random events due to mutations.

3.6.2. Subtypes H1N1 and H3N2.

The obvious seasonal aspects of the incidence of influenza A subtypes are shown in Fig. 3.12(a) and (b). As with all notifications, regardless of subtype, the beginning of each epidemic coincides approximately with the start of a new school term after the Christmas holiday.

The correlograms and spectra for the two strains (H1N1 and H3N2) can be seen in Fig. 3.13. The data used spans the years 1973 to 1985 (see Fig. 3.12). A lack of periodicity, which is not well sustained (denoted by the lack in smoothness of the curve) can be seen in the correlogram for the H1N1 subtype, when compared to that of the H3N2. The pattern observed for the H3N2 is almost identical to that seen in the analysis of the non-specific influenza A virus, in both the correlogram and the spectra. However, if seasonality is removed, as for all notifications, described above, then all periodicity is absent, shown by both the correlograms (Fig. 3.14) and the spectral analyses (Fig. 3.15).

3.7. Discussion.

The analysis of case notification data is fraught with many problems in the case of influenza. Case notification data both contrast and complement serological studies, in some ways supporting results obtained through serological analysis, but in others simply revealing the inadequacies of this method of study. The main problem with the case notifications dealt with here is that influenza is not a nationally notifiable disease. This causes the resultant notifications to be variable not only in the seasonal magnitude of the epidemics, but also leads to inter-age-group variation in reporting. The tendency will be for those age groups which are most at risk of developing complications (the two extremes of age; old and young, as discussed in detail in chapter 1) to be brought to the attention of a General Practitioner, and thus reported to the PHLS, more frequently than the age groups with a lesser risk. Even those data which are available for analysis are not as detailed as might be desired. No strain-stratified data is available for the years before 1978, nor has the data for the years 1985 onwards been published in strain specific form yet. It is also very difficult to accurately type

specific strains, since the genetic drift observed can be very slight yet still cause a new epidemic due to the new strain type. However, it is assumed that the strain typing, performed at the CDSC (by haemagglutination inhibition or single radial haemolysis), gives accurate information on the exact strains of influenza which are circulating during each epidemic season (Chakraverty et al. (1982 and 1986), Pereira and Chakraverty (1977 and 1982)).

Due to the epidemic nature of influenza, the case notifications are not representative of the continuous acquisition of immunity to the virus through time, and therefore do not represent the gradual build up of immunity with age that the notifications of endemic viruses do. As a consequence, it is not possible to build an 'age prevalence profile' for the various strains, which would be useful as a comparison to the serological work presented in chapter 5, and which would also facilitate the estimation of age-dependent forces of infection (Grenfell and Anderson (1985)). It is not possible, therefore, to derive any estimates of the basic reproductive rates for these strains, since no forces of infection can be estimated, nor do the average ages of infection represent the continuous exposure of a cohort of individuals to an endemic virus strain (as previously described). It is thus only possible to make qualitative judgements on the age-specific nature on the transmission of these virus strains from the case notification data presented here. Having made the above points, however, it is still possible to make certain deductions from case notification data.

The average ages of infection for the strains which were co-existing during the season 1982/83 (A/Eng/333/80 and A/Bel/1/81) are dissimilar to those observed for influenza A regardless of strain (somewhere between 20 and 30 years old). Both values are much lower than those found for influenza A regardless of strain, although this may be an artefact of the age grouping. The H1N1 subtype, which includes the strain A/Eng/333/80, has been circulating for a longer time than the H3N2 subtype (A/Bel/1/81) which may explain the higher average age at first infection; more immunity has been built up against the H1N1 subtype in the older age groups.

Most of the observed cases occur in the 15-44 year age group, which is in agreement with the average ages of infection of between 20 and 30 years old which have been recorded in this study and by others (Table 3.1). Further stratification of this age group is desirable to determine which elements in this group are responsible for these age effects. Unfortunately the data does not allow this analysis which would be useful to determine whether the ob-

served high numbers of infected individuals in this age group are in fact of school age. This would support the findings of Glezen (1982) and Fine and Clarkson (1982), who surmise that the observed patterns of infection are due to intensive mixing in secondary schools. However, it is clearly shown by the data (Fig. 3.7) that there is considerable disparity of the age-specific patterns of infection between different strains of the same virus. This may be due as much to the seasonal variation in mixing patterns between individuals, or simply as a result of the climatic variation between epidemic seasons, as it might be due to heterogeneity in the strain-specific infectiousness of the virus.

It appears from the cross-correlation of the case notification data that, taking into account the effect of seasonality, if H3N2 occurs in the population before H1N1, it is very likely to be followed by an epidemic of H1N1 in the same year. However, no other obvious patterns are clear from the cross-correlation analysis. The most striking feature to arise from the statistical analyses of the case notifications was the lack of any non-seasonal, long-term cycles in the oscillations of the infected (and hence susceptible or immune) populations. This contrasts strongly with the clear-cut cycles found for, most notably, measles in England and Wales, which has been found to have a regular 2-year cycle (Anderson, Grenfell and May (1985)). These long-term cycles are a reflection of population density and birth rate, caused by the periodic fluctuation of the susceptible population due to the acquisition of infection, and hence immunity, and the replenishment of susceptible individuals by new births. Therefore, in the case of influenza, which presents an antigenically 'new' viral strain every epidemic season it can reasonably be assumed that the proportion of the population susceptible to each new strain is not far off 100% in the absence of cross-immunity. If this were the case it would, therefore, have the effect of keeping the proportion of susceptible individuals (and hence infected or immune) at an approximately constant level for every epidemic season. As such, no regular cycles would be maintained for any population which was exposed to the ever-changing influenza virus. This would also be indicated by the lack of any periodicity in the average age at first infection (itself derived from estimates of the age-dependant forces of infection discussed earlier). It is useful to consider here the results from the numerical analysis of the mathematical model, presented in the previous chapter, which generated isolated and, to a degree, unpredictable epidemic recurrences of differing strains.

The seasonality of the influenza virus is very obvious (Fig 3.9.) and can readily be associated with the timing of school holiday periods as has been considered for measles (Fine and Clarkson (1982) and influenza (Glezen (1982)). It may, therefore, be the case that the aggregation and disassembly of schoolchildren is the underlying factor which precipitates the contact of infected with susceptible individuals and thus leads to the observed incidence of new strains of influenza in a population. However, if this was the case, the average age of infection could be expected to be lower than was found (between 20 and 30). This indicates that either the school system is not as responsible for the outbreaks as is suspected, or the estimation of the average age made here is too high (probably due to over-representative reporting of cases in the elderly). The latter is highly probable because of the errors inherent in the reporting system as discussed earlier.

The bi-modal epidemic curves which were predicted from the numerical analysis of the mathematical model explored in chapter 2 are reflected in the case notification reports. This suggests that these epidemic curves are caused by the co-existence of two or more strains of the same virus. In addition to giving credence to the model, the data presented in this chapter indicates that the forces of infection vary between strains of the influenza virus, and between age groups of the human host. However, in order to further define these patterns, it is essential to investigate detailed serological data. This is done in the following chapters in order to determine values for the age specific forces of infection for various strains for which data was collected.

Chapter Four: Serological Methods and Materials.

4.1. Introduction.

Infection with a virus, and hence exposure to the expressed antigen, generates the humoral immune response described in chapter 1. This may be measured using a variety of conventional immunological tests that determine levels of antibody activity. These rely on the binding properties of the antigen-antibody complex, and the ability to detect the antibody once it has bound to the antigen under laboratory conditions. It is then a straightforward matter to quantify the amount of antibody, raised against a specific antigen due to natural exposure, present in a serum sample. The level of antibody generated by an individual gives a generally reliable measure of that individual's exposure to the specific virus against which the antibodies are directed. However, because of the potential element of cross-reactivity between the antibodies under test, a positive result for antibody presence does not absolutely indicate previous exposure to infection. In theory, those antibodies which are cross-reactive should confer some protection (of unknown degree) against infection with a second, similar strain. Therefore, some individuals that show a positive result will not have been infected with the strain for which the test is specific; those antibodies which are detected will have given protection against infection with this strain as a result of previous infection with an antigenically similar strain. It is necessary, therefore, to determine what percentage of positive results are due to cross-reactivity in the test, and what percentage are due to cross-immunity between similar strains. Towards this aim certain statistical tests have been performed (in chapter 5) to determine whether the presence of antibody directed against two strains of virus in the same individuals is due to cross-reactivity, cross-immunity or consecutive infection with the two strains. However, there remains the unanswered question of whether or not cross-reactive antibodies are also protective.

Although there are a number of different serological assays which have been used in the past to measure the level of antibody raised against the influenza virus in sera (Evans and Olson (1982), Sato et al. (1988) and Julkunen et al. (1985)), it is now widely recognised that the Enzyme-Linked Immunosorbent Assay (ELISA) is the most specific and convenient to

No. of

Individuals
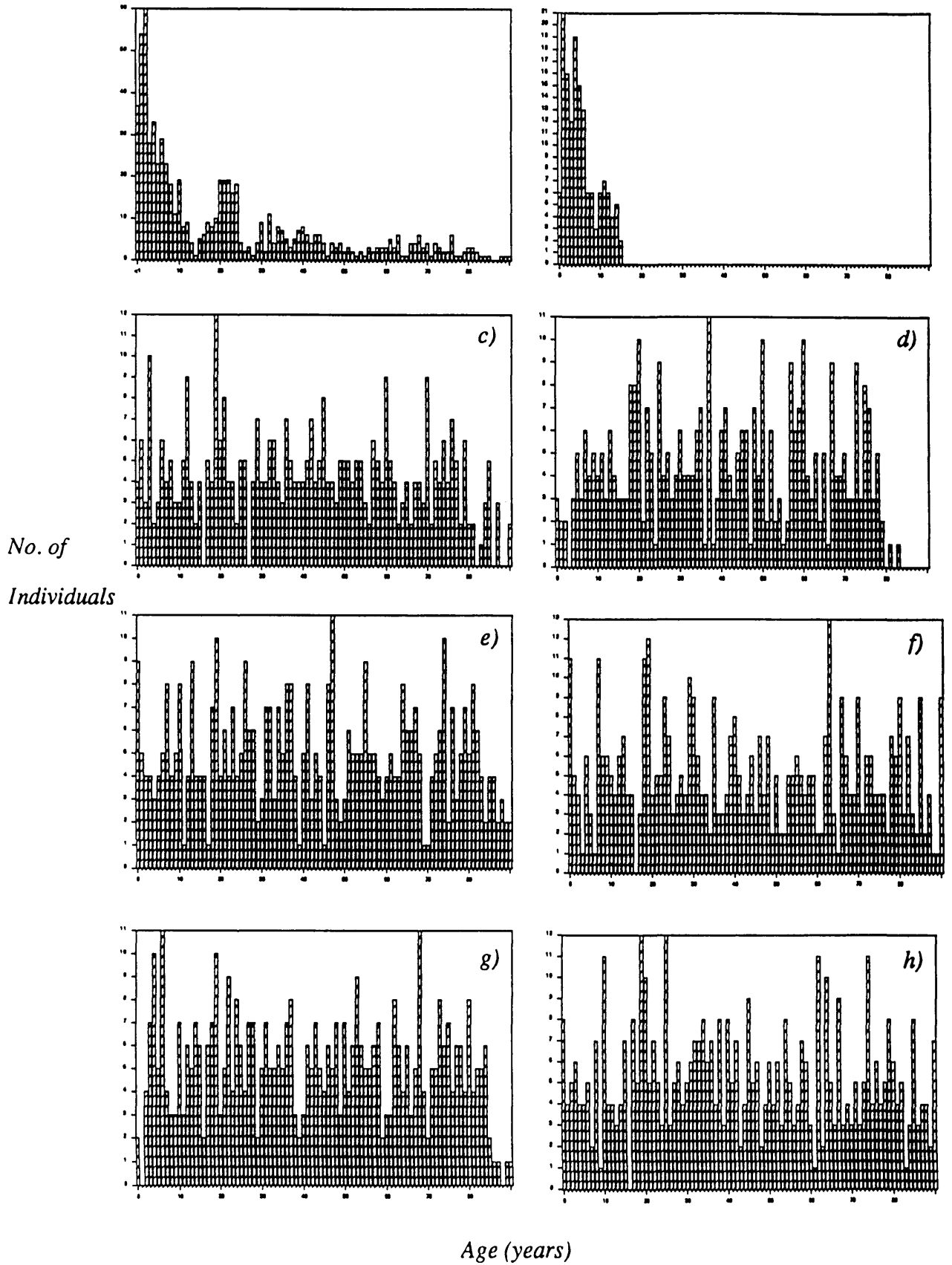
Age (years)

Figure 4.1; Numbers of serum samples in each age class used in the study. Classes are 1 per year of age, 0-90. The years the sera were collected in are; a) 1969 (n=705), b) 1973 (n=147), c) 1979 (n=387), d) 1981 (n=371), e) 1985 (n=451), f) 1987 (n=462), g) 1988 (n=464), h) 1989 (n=481).

Table 4.1. Summary of sera sets screened for the three strains of influenza A; A/HK/68, A/Bel/1/81 and A/Eng/333/80.

Year.	Number of sera in each age group.							
	0-4	5-9	10-14	15-24	25-34	35-44	45+	Total
1969	212	104	41	129	48	57	114	705
1973	74	43	28	2	0	0	0	147
1979	25	21	23	49	45	48	176	387
1981	10	23	22	50	49	48	169	371
1985	25	26	26	51	55	51	217	451
1987	27	27	26	60	51	52	219	462
1988	23	26	28	60	54	51	222	464
1989	27	19	26	62	63	54	230	481

use for rapid diagnostic purposes (Jennings et al. (1981), Julkunen et al. (1984), Koskinen et al. (1987) and Van-Voris et al. (1985)). However, this technique has not before been employed in the screening of large data sets for multiple viral antigens.

This chapter describes the preparation of the antigen; the method by which the viruses used were grown and how the antigenic determinant, the surface protein haemagglutinin, was removed and purified. The assays used to detect antibody raised against the antigens are then described, with the various modifications needed to give greater specificity; the haemagglutination (HA) test was used to quantify the antigen concentration, and an indirect ELISA was used to detect specific antibodies in the sera raised against the antigens.

4.2. Sera and Antigens Utilised.

4.2.1. Collection, storage and treatment of sera and antigens.

Sera were collected from the South Yorkshire area to achieve a regional picture of the change in antibodies raised against the influenza virus through time and across age. The samples were initially collected by the Northern General Hospital in Sheffield from various refer-

rals, including out-patients, ante-natal females, and sera sent in from general practitioners for diagnostic tests.

The characteristics of the serum samples, with the antigens against which they were tested, are summarised in Table 4.1. Throughout it was attempted to test sera from a good cross-section of age classes. Figures 4.1(a) through (g) show the age distributions for all the sera sets used. It can be seen that only the sera collected from 1969 (Fig. 4.1(a)) and 1973 (Fig. 4.1(b)) have a large proportion of the individuals sampled under 5 years of age. The years 1979 to 1989 have an even distribution of individuals in all age classes, which would appear to be ideal, but, since most of the analyses performed in chapter 5 involves the measurement of parameters which predominantly act on the younger age groups, a distribution with a larger number of individuals in the infant age classes is desirable. During analysis of the data, to overcome the problems associated with missing sera for some ages, the data were aggregated into consistent groups, each age group containing at least 10 individuals.

The sera set for 1973 is incomplete, due to the poor treatment and storage of the sera before testing. It should also be noted that in each sera set some ages are drastically under-represented.

All sera were stored at -27°C and had been heat-inactivated at 56°C for 30 minutes to remove non-specific haemolysins prior to any serological testing.

For use in ELISA tests sera were stored in a pre-diluted form using a solution of 50% glycerol and 50% phosphate buffered saline (PBS) as the solute (see appendix A for all buffer recipes). Each serum sample was diluted to 1 in 20 (i.e. 10ul of serum in 190ul of glycerol/PBS) in either a microtitre plate or a bullet tube in a microtube rack, covered and stored at -27°C. This gives a large 'stock' solution of sera for use in the serological analysis achieved using a small amount of pure sera (10ul). Because glycerol is an inert medium, which remains liquid at below -27°C, all freeze-thaw effects (which can be harmful to proteins in the sera) are avoided. The addition of glycerol is a method frequently used in maintaining activity during storage of highly purified conjugates and monoclonal antibodies (Kemeny and Chantler (1988)) and its effect on antibody potency has been assessed by Cox (1990).

The viruses used were A/Hong-Kong/68, A/Eng/333/80 and A/Bel/1/81, which were supplied by J.Skehel of the National Institute for Medical Research (NIMR). The Hong-Kong/68 was supplied as purified haemagglutinin and the A/Eng/333/80 and A/Bel/1/81 were supplied as freeze-dried whole virus enabling them to be grown in chicken eggs (see below). All viruses were stored at $-27^{\circ}C$.

4.2.2. Characteristics and limitations of the serum samples.

A central component of many seroepidemiological investigations of infectious disease transmission is the construction of data representing changes in the population with antibodies directed against the virus across a range of age classes (a horizontal cross-section). Ideally this should be based on a collection of serum samples obtained by random selection from a defined population at one point in time. Specimens should be taken from individuals over a wide range of age classes (finely stratified, with equal numbers in each age group) for whom information on sex, socio-economic class and origin is available.

In reality, practical considerations such as the availability of samples are likely to be of equal importance in determining the exact nature of serum collections used in epidemiological study. This is certainly true for the sets of samples used in this project, the details of which are given in Table 4.1. Selection of specimens was made, as far as possible, according to the guidelines outlined above. The serum utilised had already been collected for other seroepidemiological studies (Nokes et al. (1986) and Cox (1990)), and the composition of the sample was therefore predetermined. However, these criteria had been followed as far as possible when the collection was made (it should be noted here that some 3000 sera were available, and therefore practical resources, such as time and money, dictated that this existing sera be used).

The source used was a hospital virology department in the South Yorkshire area (covering Sheffield, Barnsley, Rotherham and Doncaster). Specimens had already been collected, and stored for varying lengths of time, for previous serological tests.

It is very important to mention the limitations of the collections as sources of epidemiological data:

(a) it cannot be stated, with any degree of certainty, that the surveys detailed in Table 4.1 represent random samples from the study population;

(b) they do not necessarily represent an unbiased cross-section of all social or economic classes, and probably include samples (of unknown numbers) from people whose origin or birthplace is outside the UK;

(c) the study areas include rural and urban parts;

(d) not all age classes or sexes are equally represented. Difficulty was experienced in obtaining specimens from children, particularly those less than 10 years old, and in general samples from females were more easily acquired (e.g. antenatal patients).

These points raise doubts about how representative results from the analyses are for the study area. Specimens have not been segregated according to any factors mentioned above. This is due to both the incompleteness of the records and the fact that it would induce a significant reduction in sample size within a finely stratified population.

There can be no doubt that factors such as city or country life, and perhaps social class and ethnicity will affect the transmission dynamics of an infectious disease agent (e.g. the rate of acquisition of infection) (Anderson,R.M. (1982)). Nevertheless, there was adequate justification in using these samples, because of the relative ease with which they could be obtained, as a first attempt to achieve the aims of the study. The collections of the samples shown in Table 4.1 do approximate to the requirements of cross-sectional horizontal surveys; samples were collected over short intervals of time, for a wide range of ages, and in most cases information on sex was available. The serological surveys performed serve as the basis of a 'pilot study' and achieve the broad aims of the project which should stimulate the interest in, and recognition of the importance of, further serological surveys in the epidemiological research of influenza.

4.3. Growth and Purification of Virus Strains

The strains of virus which were used as antigen in this study were chosen as being representative of any epidemic season during which two strains, each of a different subtype, were circulating. In this case the two strains co-circulating were A/Eng/333/80 (H1N1) and A/Bel/1/81 (H3N2). These were both present during the epidemic season of 1982/83 (Chakraverty et al. (1986)). The sera were also screened against A/Hong Kong/68 (H3N2) (which

was first detected in 1968 (Pereira and Chakraverty (1977)), to examine any possible inter-actions between the virus strains from each season.

4.3.1. Growth of Viruses.

The method used for the culture of influenza viruses was that of Grist et al. (1979) who state that the 'Development of routine cell culture methods has reduced the importance of eggs but they are still valuable for the ... isolation of influenza virus. Egg inoculation is more sensitive than RMK [Rhesus monkey kidney] cell culture for most [influenza] type A strains.'

Approximately 600 eggs were incubated at 37-38°C with 40-70% relative humidity and turned twice daily for ten days. On the 11th day they were sorted by shining a bright light into the interior of the egg in a darkened room, a process known as 'candling'. Those eggs which were still viable were further incubated for a few hours before inoculation with virus (as described below), while those eggs which were unfertilised or dead were discarded. The viable eggs which showed satisfactory development of chorioallantoic blood vessels and embryonic movement were marked with pencil to indicate the limits of the air-sac (which enlarges further during subsequent incubation) and a smaller mark was made on the side which showed the best developed vasculature, taking care to mark away from any veins.

The freeze-dried seed viruses for each strain were independently suspended in 1 ml of filtered, double distilled water. These solutions were then made up to 60 ml. with PBS con-taining a small amount (approximately 1%) of antibiotic.

Two small slits were then drilled over the chorioallantoic membrane (using a rotary drill), one just above the air-sac to allow pressure to be released, and the other on the small mark near, but not over, a vein. The second slit was then wiped with a spirit swab and 0.5 ml. of the seed virus injected into the allantoic fluid through a thin 1.5 cm long needle. Both slits are then sealed with a mixture of paraffin wax and petroleum jelly. Each strain was injected into approximately 270 eggs, that being half the number of all the viable eggs.

The eggs were returned to the incubator, with air-sacs uppermost, for 3 days, after which time they were chilled at 4°C for 12 hours to ensure that the embryo was dead before the al-lantoic fluid was drawn off.

To harvest the virus the shell was removed from the air-sac, after swabbing the area, and the allantoic membrane was broken using a Pasteur pipette, which was then used to aspirate the allantoic fluid. The total amount of allantoic fluid removed from each batch of 270 eggs was 2.25 litres. During the harvesting procedure every 30 eggs were pooled together and a spot haemagglutination (HA) test (detailed in section 4.4.2) was performed to determine the concentration of the harvested virus before it was centrifuged; all those that gave good haemagglutination unit (HU) values (greater than 1/1000) were spun at 2000 G for 20-25 minutes to remove cell debris.

The supernatant was carefully poured off and then further centrifuged at 30000 G for 150 minutes to pellet the virus. The supernatant from this spin (which had an HU of less than 1/128, representing a viral yield of 80-90%) was discarded and the pellet resuspended in PBS. This procedure was repeated several times which eventually gave 20 ml. of each strain of virus with an HU of 1/12 showing the viral concentration to be in excess of 1 mg/ml.
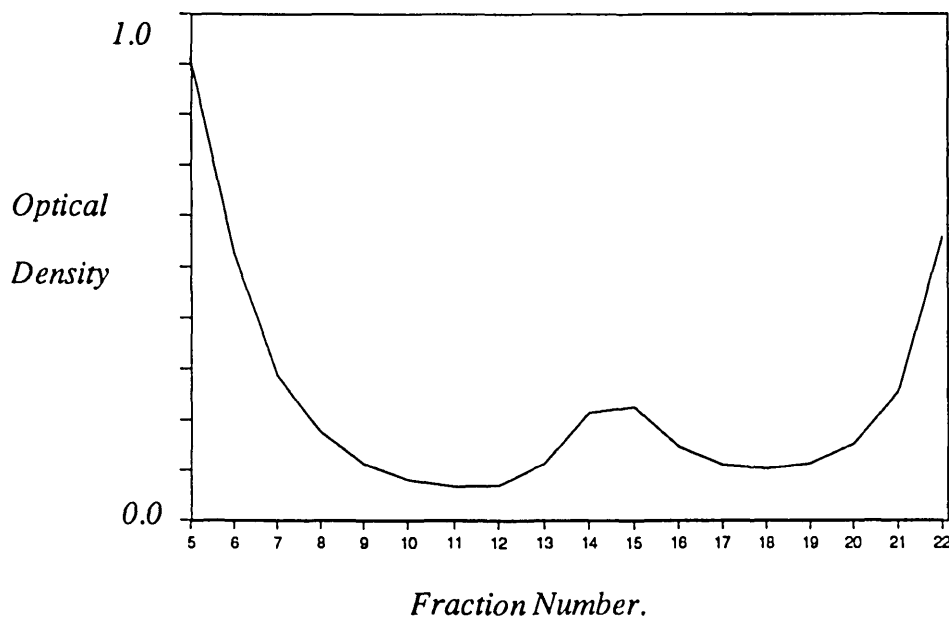
Figure 4.2; An example of the fractions of the digested influenza virus proteins separated from the sucrose density gradient. The peak at approximately fraction 13 is caused by the haemagglutinin protein.

4.3.2. Purification of Haemagglutinin.

The method used was that described by D. Stevens of the National Institute of Medical Research (pers. comm.) and known as the 'Bromelain HA-Method'. Initially 30 mg of EDTA and 287 ul of B-mercaptoethanol were added to 100 ml of Tris/Cl buffer (see Appendix A for details), and allowed to stand for several hours, ensuring that the B-mercaptoethanol had fully dissolved. 100 mg of Bromelain powder was then added, 3 ml of the resulting solution was mixed with an equal volume of concentrated virus and the Bromelain/virus mixture was incubated at $37^{\circ}C$ for 4-6 hours.

After this time the supernatant was removed and layered on to a continuous (5%-25%) sucrose density gradient. The gradient was spun for 18 hours at 35000 rpm at $15^{\circ}C$, and the fractions measured in a spectrophotometer at 280 nm. A typical result from the spectrophotometer is shown in Fig. 4.2, where the peak in the absorbtion spectra due to the HA can clearly be seen approximately 2/3 down the SDG. These fractions were kept for use in the ELISA's and the others discarded.

4.4. Serological Assays.

4.4.1. Protein Concentration Determination (Bradford assay).

To test the concentration of the resulting HA protein, for use in the ELISA's, the standard Bradford assay was used (Bradford,M.M (1976)). A double dilution of BSA (4 mg/ml to 30 ug/ml) was made up in a microtitre plate, and the test samples were placed in separate wells of the plate, all in volumes of 50 ul. 150 ul of the Bradford reagent (see Appendix A) was added to each well containing protein (BSA or HA) and allowed to equalise. The resultant colour change was then recorded by a plate-reader at 620 nm. By comparing the test sample of HA to the standard curve created by the double-diluted BSA it was possible to determine the concentration of the HA for each strain.

4.4.2. Haemagglutination Assay (HA).

a) Introduction

Certain viruses (the myxoviridae) possess the ability to cross-link red blood cells (RBC) by the antigens expressed on their surface (in the case of the influenza virus the antigen is haemagglutinin - named after this very property). A haemagglutination titration of viral

antigen defines the haemagglutination unit (HU) for that virus; this is the reciprocal of the highest dilution of viral antigen at which an agglutination reaction occurs. An HA value of 1 HU is considered to represent the optimal antigen concentration to be used in a serological assay, since it represents the highest dilution which exhibits a complete haemagglutination reaction (Conrath and Coup (1978)).

b) Method

A volume of 0.2 ml of virus sample was double-diluted through 0.2 ml of PBS in a haemotitre plate, and 0.2 ml of 0.5% clean fowl red blood cells (RBC's) added to each of these wells (the RBC's had previously been washed three times in phosphate-buffered saline, centrifuged, and resuspended). The mixtures were incubated at room temperature for approximately 20 minutes and then examined. A negative result was recorded if the RBC's were clumped in a pellet at the bottom of the wells, and a positive result recorded if the cells were suspended in a dense mat (indicating agglutination). From the double-dilution it was possible to determine the lowest titre at which a positive result was obtained for the test solution containing the virus.

4.4.3. The Enzyme-linked Immunosorbent Assay (ELISA).

a) Introduction.

Techniques were developed for screening all sera involved in the study for IgG antibodies specific to the influenza strains used. The methods used were based on the ELISA theories of Voller and Bidwell (1976). The system was developed so that all tests were carried out using identical equipment and reagents (which were all used at the same concentrations) with the specific antigen being the only variable component of the assay. Even the concentrations of antigen (in mg/ml) were constant throughout the tests.

The antigens used were those grown from the seed viruses in the manner described above in section 4.3.1.

The first step in the development of the procedure was to determine the optimal concentrations of antigen and test serum to use in the assays. This was achieved by using a system called 'checkerboard titrating' of antigen against sera which were considered to be either positive or negative (Voller, Bidwell and Bartlett (1980)). The definition of a true positive,

or a true negative, has been investigated with some rigour in the study of parasitic infections. Yerushalmy (1947) developed the concept of specific diagnosis by defining the concepts of sensitivity and specificity for a diagnostic test. By his definition, the sensitivity of a test is its capacity for making a correct diagnosis of an infected individual, while the specificity of the test is its capacity for a correct diagnosis in a confirmed negative case. Feinstein (1975) further developed this concept by using two-way tables to quantify the specificity and sensitivity of a test. However, it is obvious that these definitions can have no realistic meaning in the context of this study, where no validation of infection can be made. In fact the tests performed, considering that the antigens were all purified specifically, constitutes the only means of determining whether an individual has been exposed to the virus strain or not (taking possible cross reactivity into account). However, the concept of determining whether there are more or less positives than would be expected, and hence whether there is cross immunity between strains, or cross reactivity in the test, can be examined in a similar manner. This is dealt with in section 5.9.

Unfortunately no known negative or positive sera were available, nor was a standardised test, due to the specific nature of the viral antigen used. The positives and negatives used for the standards were, therefore, selected by random screening of some 500 samples to obtain a number of sera with high and low titres respectively. Before the samples were selected for use as standards it was determined that the high titre sera were from individuals in the 65+ age group (and, therefore, most likely to have experienced infection), whilst the negatives were all taken from infant samples (and therefore less likely to have been exposed to the virus strains). There are obvious limitations to this method of selecting positive and negative sera; 'positive' sera may well contain cross-reactive antibodies, as mentioned earlier, which indicate a positive result where one does not exist; while 'negative' sera, on the other hand, may contain passively transmitted maternal antibodies to either the strain under test, or to an antigenically similar strain which was circulating previously. However, without classified 'positive' and 'negative' sera for use in the test, it is impossible to adequately standardise the procedure, so, taking the above points into account, some randomly selected sera were used as positive or negative controls, dependent on the antibody titre levels.

Antigen Concentration (ml).
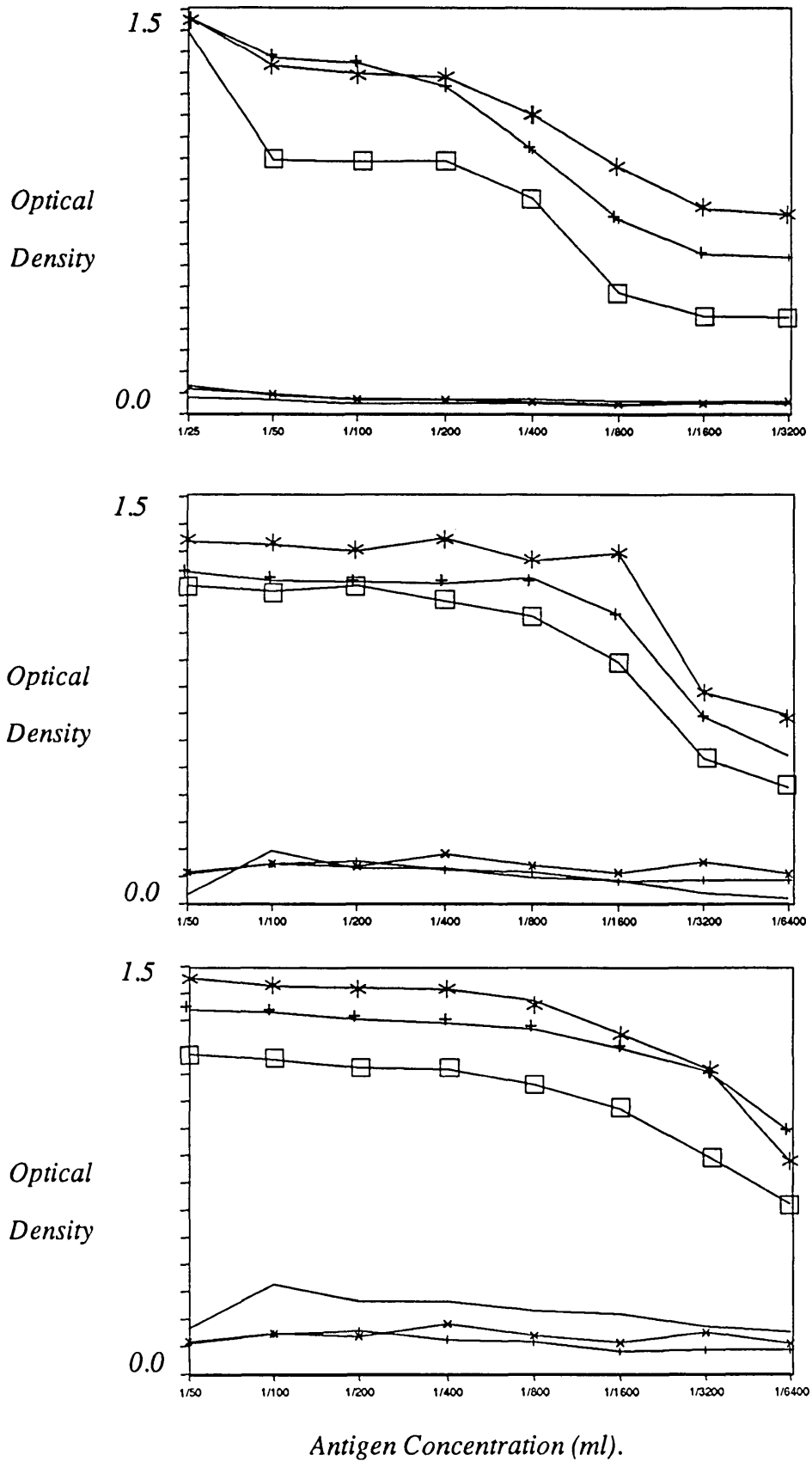
Figure 4.3; The concentrations of sera titrated against differing concentrations of antigen in the checkerboards for; a) A/Bel/1/81, b) A/Eng/333/80, c) A/Hong Kong/68. Positive sera are represented by -*- (1/100), -+- (1/200) and - - (1/400). Negative sera are represented by the lower three lines (at the same concentrations).

b) Procedure for the ELISA Checkerboard.

The procedure used was consistent for all three antigens, with only the known high positive and negative sera differing, these having been found on each occasion by the random screening of 500 sera. The sera were known to have been collected after the epidemic caused by the antigen under test, which was used considerably in excess for the random screening. In this way it was possible to find negatives and high titre positives specific for each antigen, which could then be used for the checkerboard itself.

Initially, for the titration, the antigen was diluted in carbonate 'coating' buffer (pH 9.6) to 1 in 25 and 100ul of the solution was added to each well in row A of an enzyme-immunoassay microtitre plate, previously prepared with 100ul of coating buffer in every well. The antigen was then double-diluted in the wells down the plate through rows B to H, giving a final range of dilutions from 1 in 50 to 1 in 12800. The plate was placed in a humidifying chamber at 4°C overnight to allow passive adsorption of the antigen onto the plate.

After incubation the plates were washed twice with washing buffer, each wash taking approximately 30 seconds, and excess fluid was then shaken away. This was followed by the blocking stage using 5% bovine serum albumin (BSA) as the blocking protein, dissolved in 100ul PBS, which was incubated at room temperature for one hour. The purpose of blocking with a non-specific protein like BSA at this stage is to prevent subsequent adsorption of non-specific antibodies or conjugate to the plate.

The plates were washed twice for a second time after blocking, and 100ul of incubation buffer were then added to each well of the plate. The positive and negative sera previously determined by random screening were then added to columns 2 and 8 of the plate respectively at a concentration of 1/25 and diluted across the four other columns of the plate, leaving columns 1 and 7 for use as 'antigen-only' controls, thus giving a range of dilutions from 1/50 to 1/800 for both the positive and negative sera. The plates were incubated at room temperature for 3 hours to allow any specific antibodies present to bind to the antigen in the wells.

The third wash of the plates was performed three times and 100ul of the conjugate solution consisting of goat anti-human IgG (lambda chain specific) conjugated to horse-radish peroxidase at a dilution of 1/2000 was then incubated in each well for 3 hours. This dilution

was chosen by reference to other ELISA's for the detection of viral antigen in which the same concentration was used (Nokes,D.J. et al. (1986), Cox,M.J. (1990)).

Freshly made 'working' substrate solution (see Appendix A for details) was then added to the wells in 100ul amounts after the final washes (three times), and the peroxide reaction was allowed to proceed for 20 minutes before being stopped by 50ul of 2M sulphuric acid. The plates were then read at 492 nm in a spectrophotometer to assess the optical density (O.D.) of each well.

Typical examples of the checkerboard results are presented in Figs. 4.3(a), (b) and (c), where it can be seen that all antigen concentrations are in excess at 1 in 400. Low optical densities were obtained for all negative sera, and a significant demarcation can be determined between the positive and negative sera. The optimum antigen concentration was chosen to be 1.5 ug/ml (the concentration of the antigen at a 1:100 dilution) and the optimum sera dilution was found to be 1/200. At these dilutions, neither the antibody, nor the antigen, were in considerable excess, although it can be seen that the antigen is on the plateau of the concentration curve. It is important to ensure that, while the antigen may be in excess for the test, the concentration of sera must be kept at a low enough level to prevent any saturation of the antigen; i.e. a dynamic equilibrium must be maintained between the two.

c) Refinements to the ELISA Technique.

Once the optimum concentrations of antigen and sera were determined it was possible to refine the procedure to give maximum separation of positive and negative and minimum background. Initially, all reagents were titrated below and above those concentrations used in the checkerboard. Foetal calf serum (FCS) was also considered as a substitute for BSA, but was found to make no discernable difference.

Next, a number of different makes of plates were tested (Linbro EIA I and II, Nunc and Dinatec) to determine which had the most suitable properties (i.e. maximum antigen adhesion, minimum non-specific protein adhesion). The plate which gave the clearest demarcation between positive and negative sera, with minimal background was found to be the Linbro EIA I, which was subsequently used in all ELISA procedures.
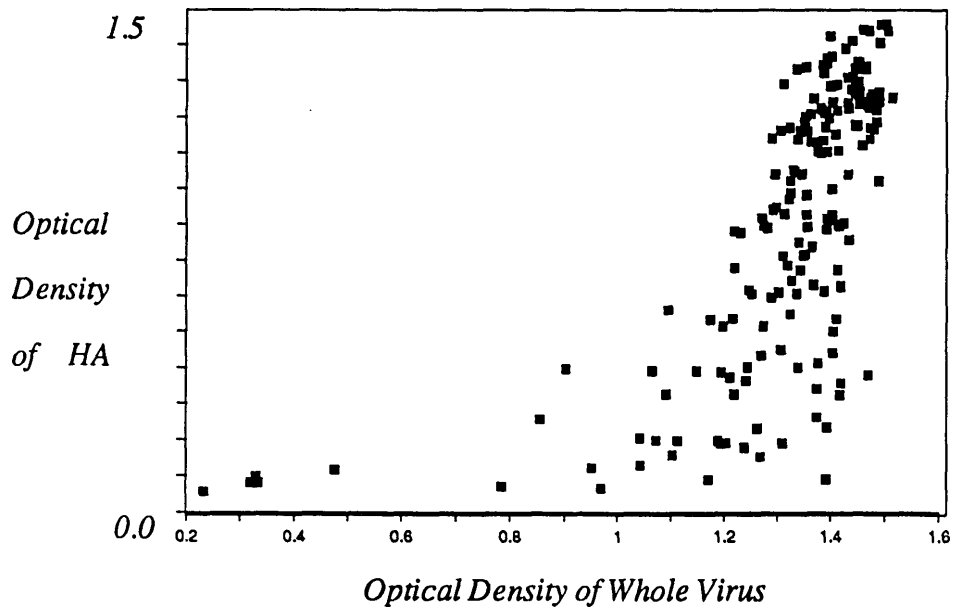
Figure 4.4; A comparison of the results obtained when screening sera against whole virus and the results obtained when screening against purified haemagglutinin for the A/Eng/333/80, using the sera set collected in 1985. The units are absolute optical densities.
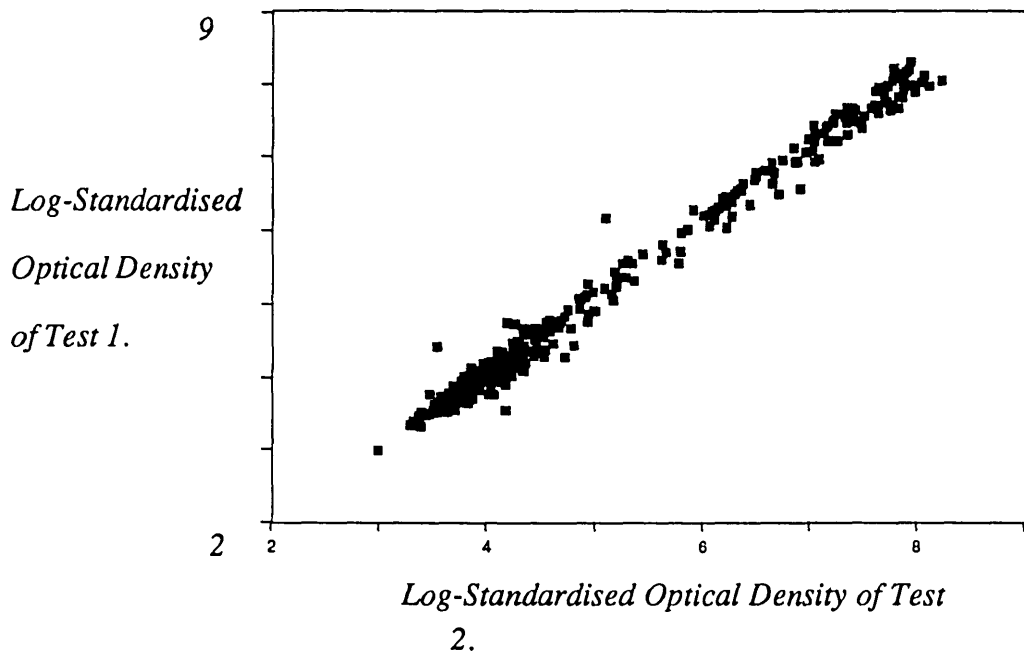
Figure 4.5; An example of a bivariate plot generated by screening the same sera set against one antigen in duplicate. Data is from 1969 screened against A/Hong Kong/68. Units are log-standardised O.D. readings as detailed in the text.

In addition to the minor modifications mentioned above, monoclonal antibodies were introduced into the procedure, both at the serum level, and at the conjugate stage. These were obtained from Dr. John Skehel at the National Institute for Medical Research in Mill Hill, London, where they were raised against the A/Eng/333/80 and A/Hong Kong/68 strains in rabbits. The monoclonal antibodies were used to attempt to capture the antigen, when it was in an impure form, and thus ensure that the test was more specific. This was done by initially coating the plate with a specific monoclonal antibody to the viral strain being used (at a concentration of 1/200). The standard ELISA technique described above was then performed. However, it must be noted that this in itself did not improve specificity for the whole virus, and therefore the HA was purified as described in section 4.3.2. With the pure HA no monoclonals were necessary as the test proved very specific. This can be seen in Fig. 4.4 which shows that the same sera tested against both whole virus and purified HA gave different results, with more negative sera recorded against the purified HA than against the whole virus. This indicates that when the ELISA technique was performed using the whole, unpurified, virus a number of false positives were recorded. The idea of monoclonal antibody capture, using the whole virus, was therefore discarded and the purification of HA from the virus pursued. No monoclonals were used in conjunction with the haemagglutinin; due to the purity of the protein (see Fig. 4.2) it was considered to be unnecessary.

During the conjugate stage, however, monoclonals were used to very good effect, in the following manner. After the plates were washed, following the addition of sera, instead of adding the anti-human conjugate a monoclonal antibody, raised against human IgG antibodies in mice, was added, thus ensuring that when the anti-mouse conjugate was added only the IgG constant chains of the human sera were labelled, thereby adding greater specificity. The monoclonal was used at a concentration of 1/1000, and the anti-mouse conjugate was added after one hour (following three washes) at a concentration of 1/2000.

Monoclonals were also used in a less successful context, when the method of competitive ELISA's was considered. Due to the lack of success using this technique nothing more need be mentioned about it here.

Initially, every serum sample was tested in duplicate to ensure that no inconsistencies were occurring in the test (see Fig. 4.5), but since the level of error between duplicates was very low (r=0.994, P>0.001; n=344), and a good separation could be seen between high and

low O.D. readings, it was decided to continue screening sera singly, to save both time and sera (which was in relatively short supply).

All details of buffers and equipment used in ELISA's are given in Appendix A.

4.4.4. The Standardising Procedure.

Positive sera, determined by previous random screening (discussed in section 4.4.3(a)), were serially diluted two-fold from neat to 1/256 in predetermined negative sera prior to dilution in the glycerol/PBS mixture, and stored at -27°C with the test sera. This allowed a curve to be fitted between the doubly diluted positives giving a linear relationship between the log-values of the diluted positive sera and an arbitrary 'standard' scale. The calibration of the optical density readings for the test sera on each plate can then be performed, by comparison with this standard curve, if the double-diluted 'standard' positives are included on each test plate. The following algorithm represents the relationship between the standardised values and the optical density readings, and can, therefore, be used to calculate a log-standardised value directly from the O.D. reading;

$$ S = \log_e \frac{1}{640.(2^{(v-c)/m})} $$

where;

S = the standardised value

v = the optical density of the test sera

c = the intercept of the standard curve

m = the gradient of the standard curve.

4.4.5. Screening Test Samples.

Plates were coated with HA for the appropriate viral strain, at a concentration of 1.5 ug/ml which had been determined by the checkerboards to be the optimum concentration, and then incubated at 4°C overnight. The plates were then washed and blocked with BSA for one hour. The prediluted stock sera (including both the test samples and the standards) were allowed to reach room temperature gradually and then were thoroughly mixed before
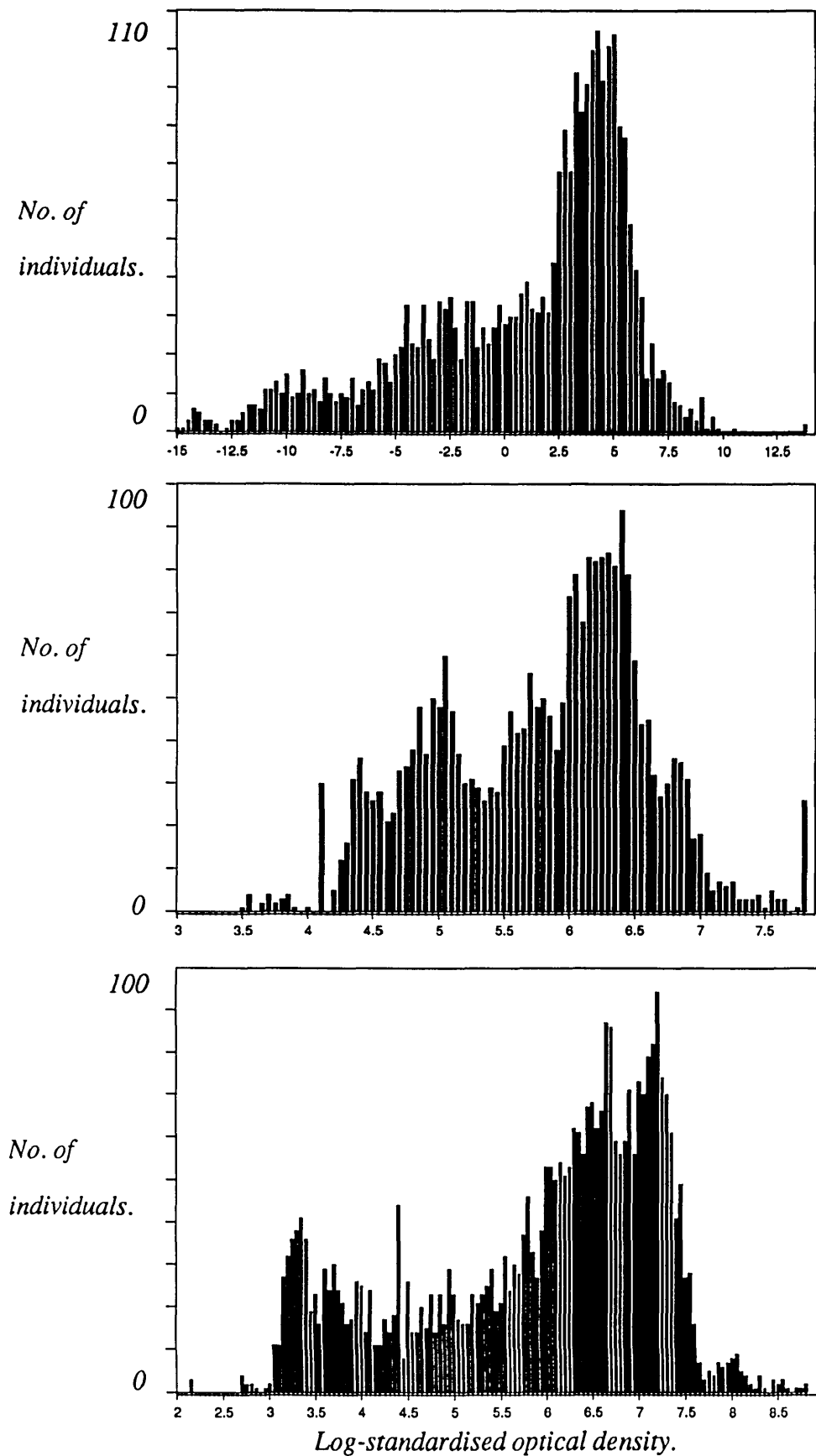
*Figure 4.6; The distribution of antibodies detected in all sera screened against; a)
A/Bel/1/81, b) A/Eng/333/80 and c) A/Hong Kong/68, for all years. Units are log-stand-
ardised O.D. readings. The normal distributions of the positive sera (high values) can
be seen to cross over the normal distributions of the negative sera (low values) in some
cases. These are considered to be the 'cut-off' points for each virus strain.*

Table 4.2. The log-standardised optical density readings used as cut-off points between positive and negative sera. All O.D. values below the cut-off points were considered to indicate that the individual had not been exposed to the viral strain.

Strain of influenza A.	Cut-off point (log-standardised).
A/HK/68	5.60
A/Eng/333/80	5.35
A/Bel/1/81	0.00

use. After washing the plates, 10 ul of stock sera were added to 90 ul of incubation buffer in each well, giving a final dilution of 1/200. In this way dilutions of test sera, standards and controls were included in each plate.

After incubation of the sera for three hours, the remaining stages were performed as with the checkerboard titrations, with the concentration of conjugate at 1/2000 and the mouse monoclonal at 1/1000. The plates were developed for 20 minutes before being stopped with acid, as detailed earlier, and the O.D. values read at 492 nm in the spectrophotometer.

The distributions of antibody concentrations for the three strains over all the years from which sera were collected are shown in Fig. 4.6, where two overlapping distributions can be seen in some of the figures. These two distributions represent the negative sera (with low O.D. readings), and the positive sera, found to contain antibodies raised against the strains (with a higher average O.D. reading). Although the cut-off points (which determine the differentiation between positive and negative sera tested against the antigens) are not always clearly defined, it is possible to estimate a standardised, working cut-off value by eye. Standardisation procedures are notoriously difficult to perform on antibody levels, unless there are internationally accepted positive and negative sera. Since these antigens were purified from whole virus no such standards are available. To enable a working relationship, those distributions with a clear cut definition between positive and negative were used as a guide for those distributions which were not so obvious and for the amalgamated distributions of each specific strain (4.6). The cut-off points were then used throughout the categorisation of positive and negative individuals for the specific strains. It must be remembered that without a method for discriminating between positive and negative individuals, the serology is
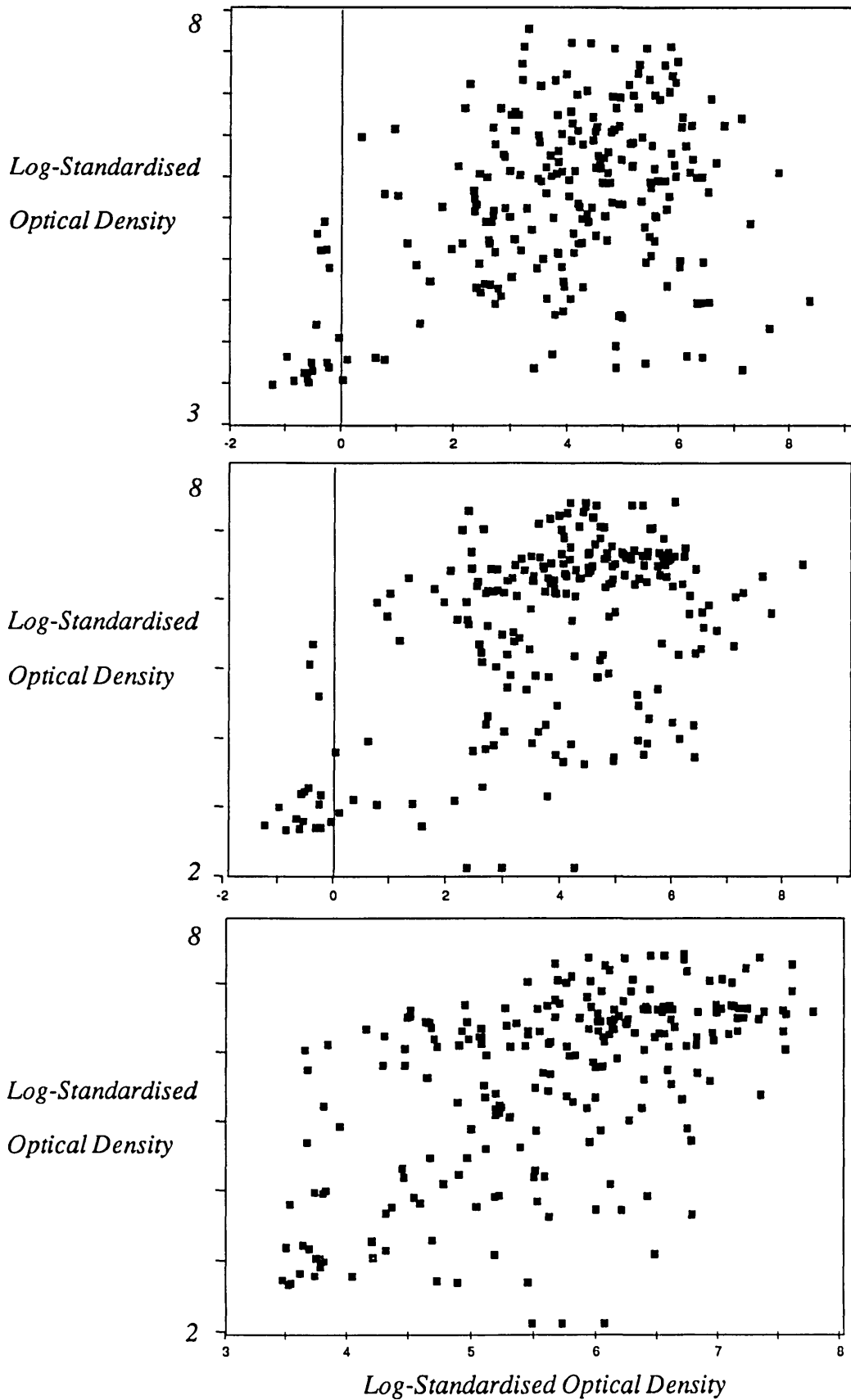
Figure 4.7; Comparisons of the results of sera screened for the three strains of influenza from the same sera set (1985); a) A/Bel/1/81 against A/Eng/333/80, b) A/Bel/1/81 against A/Hong Kong/68 and c) A/Eng/333/80 against A/Hong Kong/68. All units are log-standardised O.D. readings.

essentially worthless, so it is important to make some assumptions which enable decisions to be made. Various cut-off points were considered for A/Hong Kong, but although there were qualitative differences in the results, no significant difference was observed between the serological profiles resulting from the different cut-off points. Therefore, this was not performed on the other strains, nor was it considered necessary to do further analysis on the various cut-off options.

Because of the system of standardisation used, once a cut-off point has been determined for one strain it holds true for all sera tested. The cut-off points used for each antigen are listed in Table 4.2.

To ensure that individuals did not possess identical antibody levels for all three strains (i.e. the same antibodies in each individual were being detected by the three separate assays) comparisons were made, for each serum sample tested, between the antibody levels specific for each strain of virus. These can be seen in Fig. 4.7, which clearly shows that there is a disparity between the levels of antibody, generated by an individual, against each strain. Although this does not exclude the possibility of cross-reactivity amongst the antibodies, it does indicate that each assay is reasonably specific to each strain, and hence the antigens. Further statistical analysis is performed on this data in chapter 5 to determine the exact nature of the 'shared' antibody observed in Fig. 4.7.

4.5. Discussion.

Initially the generation of appropriate antigens for the determination of potentially cross-reactive antibodies in the test sera raised many problems. However, once the process of purification of HA was undertaken it did not prove to be an overly large hurdle. The main problem was to produce enough antigen to screen all the sera involved. This was eventually done by growing the two strains in 600 chicken eggs, which produced some 5 litres of allantoic fluid, from which in turn was harvested 20 ml of each concentrated virus. From this concentrated virus it was possible to purify 6 ml. of HA for each strain, which could be used, albeit only slightly diluted, for the ELISA tests. Once the purity of the antigen was ascertained it was possible to screen the sera, with reservations on the accuracy of the test.

The sera set which was analysed for this study was by no means ideal, and the various shortcomings have been listed in detail. The most relevant deficiencies involve the under-

representation of younger individuals compared to the needs of the analysis. The counter-weight to these deficiencies was the ease of access to the sera set, and the comparatively large numbers of sera taken from the same area over a long time interval. As a consequence some 8700 tests were performed on the various sera against the three antigens, which gives a good serological data base for the preliminary investigations of co-existing virus strains. Suggestions for the structure of the sera sets to be used in this type of work in the future are outlined in chapter 7.

The assay chosen to perform the tests, namely the Enzyme-Linked Immunosorbent Assay, was one which has been used successfully on many occasions (Jennings et al. (1981), Kemeny and Chantler (1988), Evans,A.S. (1982), Nokes et al. (1986) and Cox (1990)). Not only does it give specific, qualitative results it is also easy to perform on large numbers of sera and is very reliable. An optimised ELISA strategy for testing against the influenza HA antigens was obtained by a simple trial and error procedure. All variable components of the assay were used in differing amounts and the best combinations (i.e. those which gave optimum differentiation between known positive and negative sera, with a minimum of background) were used in the final screening of sera. The results were also standardised in each microtitre plate to prevent variability between tests and thus make all results comparable.

The indirect ELISA was chosen because of the consistency in the results obtained by this method, and hence its reliability and reproducibility. Although it is possibly not the best assay available to test specific virus strains, the antigen used was highly purified, thus making this aspect a relatively low priority in the test chosen, compared with the practicality of screening such a large number of sera. A competitive ELISA using monoclonal antibody to bind with the antigen in opposition to the sera antibody would have given a greater specificity, but a reliable protocol could not be achieved so this assay was abandoned.

Other problems with this type of screening include the definition of positive and negative values for the individuals under test, once the assay has been conducted on a sera set. This was performed by a rudimentary method of estimation to determine a suitable cut-off point between the two distributions. This method has been used successfully in the past (Nokes et al. (1990) and Cox (1990)). Other methods which might have been used to determine the cut-off point, while appearing more accurate, introduce a new element of error. These methods include the assumption of normality in the distribution of the positive and

negative sera, which is not strictly true. Either a normal distribution can be assumed and a best-fit line determined by the process of maximum-likelihood, taking the cut-off point to be at the area of cross-over between the two distributions, or the variance and mean can be calculated for the distribution of the negative sera, and an arbitrary point taken some 2-3 standard deviations from the mean, taken to be the cut-off point (Nokes,D.J. pers comm.). Both of these are reliant on the assumption of normality in the distribution, and both have an abstract method for choosing the cut-off. Some element of doubt will always remain with any method when the distributions of positive and negative sera are as unclear as they are in this study, but significant weight is added to the validity of the method used, however, by the results in the following chapter, which show a greater number of negative individuals in the younger age classes than in any other. This indicates that the cut-off points used were representative of the correct antibody level. There is no essential need to get absolute values for positive and negative, so long as consistency is observed. In an assay such as this, which is very qualitative, the main objective is to get results that are comparative, not necessarily absolute. It is impossible to achieve perfection in this situation, because there is always a certain number of both false positives and negatives, which cannot be detected.

Although the problem of cross-reactivity was dealt with as extensively as possible, the fact cannot be ignored that some cross-reactivity between the serum samples will occur, considering the nature of the antigens being tested. Test specificity is impaired because the antibodies that lead to cross-immunity will, most likely, be cross-reactive to the various antigens. The problems involved with cross-immunity and specificity can only be considered theoretically until a greater understanding of the mechanisms behind the immunity generated to different strains of influenza is achieved. Until that time it will be necessary to analyse the results from studies of this type empirically and treat with caution any conclusions regarding cross-reactivity between strains and the concept of cross-immunity. The antigens used were as distinct as possible, to a large extent eliminating the possibility of non-specific cross-reactivity. Further examination of potential cross-reactivity in the screening process is conducted in section 5.9.

It is possible to conclude that, given the quantity of sera which was tested, the most accurate test possible was performed to screen the sera in a controlled way against purified and specific antigens.

Chapter Five: Seroepidemiological Analysis.

5.1. Introduction.

Serum samples from a number of years were screened for the presence of various influenza A haemagglutinin-specific antibodies using the ELISA technique (see chapter 4). The empirical data thus provided form the basis of the seroepidemiological analyses presented in this chapter

The epidemiological interpretation of serological data involves arguing backwards from endpoints to some component of the process such as prevalence of infection, intensity of transmission or the duration of the infectious agent in the population. Interpretation of the results is always dependent on the relationships between the immunodiagnostic endpoints and the antecedent epidemiological variables of interest, taking into consideration possible confounding variables. The objectives of this study include an attempt to detect antibodies which are circulating due to either present infection or past exposure to infection in order to determine whether there is a particular influenza strain circulating at a given time. If there is a particular strain circulating, the level of seropositivity (i.e. the proportion of the population with antibodies in the sera directed against a specific strain) at this time must be determined, and whether or not this level is changing.

Serological population surveys can be one of three types; a single cross-section by age of the population; repeated cross-sections by age; or true longitudinal surveys of a cohort of individuals (where the individuals are followed through time and hence over age). In all cases the data must be recorded with respect to the age of the individual. The first part of this chapter deals with the analysis of seroepidemiological data for influenza based on a number of horizontal cross-sectional surveys in Sheffield for the years 1969, 1979, 1981, 1985, 1987, 1988 and 1989. When analysing a cross-sectional study, if there has been no transmission for a number of years, there should be no seropositive individuals below the age equivalent to the consecutive number of years in which transmission has occurred. The intensity of transmission can be estimated using a catalytic infection model which assumes a constant risk of infection for each age group. If the risk (or force) of infection has changed

over a lifetime, the model can still be used for young individuals to estimate the recent intensity of transmission. The shortcomings of a single cross sectional survey are that it does not take account of seasonal patterns in transmission, nor do the proportion of seropositives give an indication of longitudinal changes in intensity of transmission.

As a consequence of the cross-sectional surveys having been conducted on sera that were sampled over a number of years it is also possible to consider the changes in the population through time, or 'longitudinally'. A repeated cross-sectional study gives an indication of any changes in transmission between the various surveys. However, when making estimates of the force of infection from the changes in the proportion seropositive it is important to consider the changes with respect to age and time. The intention is to obtain information on the state of immunity to the influenza virus across a wide band of age classes in the population (from children less than one year old to the elderly), and to determine what impact (if any) the cross-immunity conferred between viruses has on the levels of immunity observed.

The epidemiological status of the various strains (i.e. whether they are endemic or, if not, when they were introduced into the community) is also considered in longitudinal studies of different cohorts of individuals. Longitudinal surveys of a cohort of individuals give the best data for this type of analysis, but in general these studies are impractical in terms of time and effort (i.e. they require repeated surveys each year over many decades).

The second part of the chapter considers the epidemiological parameters that can be estimated from profiles of immunity with respect to age, most notably the 'force of infection' and the average age at first infection. Conclusions drawn from the observations made in this chapter are very dependant on data collection methods and the method of analysis employed. Some comment has been made in the earlier chapter on the limitations of the sera set used and the method of collection of these samples, on the populations from which they were drawn, and on possible limitations of their use in epidemiological research.

Part I: Age-Serological Profiles.

5.2. An Introduction to age-serological profiles.

In chapter 4, the serological methods used to screen the sera for influenza strain-specific antibodies were described. The results obtained from the serological screening of the samples have been used to construct serological profiles which describe the distribution of immunity to the three strains under consideration, with respect to age. Such profiles reflect age-related trends in the rate of acquisition of infection, and hence immunity (Anderson and Grenfell (1986)), and provide an estimate of the transmission potential of each strain in the population. Age-serological profiles from horizontal cross-sectional surveys can yield information on the likely epidemic nature of an infection (Nokes et al. (1986) and Anderson et al. (1987)) and the effect (if any) of cross-immunity between directly transmitted viral strains.

5.3. Horizontal Studies of Age Serological Profiles.

5.3.1. Observations on the Immune Proportion of the Population.

When describing the appearance of serological profiles it is helpful to use specific phrases to imply the current state of immunity to a virus strain. In this thesis, the following definitions are used. The term epidemic is used to describe an exceptional number of cases over a given (short) time period. Conversely an endemic situation is one in which the virus has reached a certain equilibrium, such that the introduction of new cases each season does not significantly affect the pattern of the serological profile. Where a virus strain is described as being incident in a particular season, it implies that no cases of that strain (or no seropositive individuals) have been recorded before that year.

The Age-serological profile derived from the 1989 sera set after testing for strains A/Bel/1/81, A/Eng/333/80 and A/Hong Kong/68 are shown in Figs. 5.1(a), (b) and (c) respectively. The year 1989 was chosen as representative because it was the latest year to be screened, and therefore the data is most likely to detect the presence of endemic strains. It should be noted that A/Bel/1/81 in particular may well not be endemic, but still epidemic, at this time. This is considered in greater detail in section 5.4.

a)

Percentage of

Population

Seropositive

b)

Percentage of

Population

Seropositive

c)

Percentage of

Population

Seropositive

Age Group (Years).
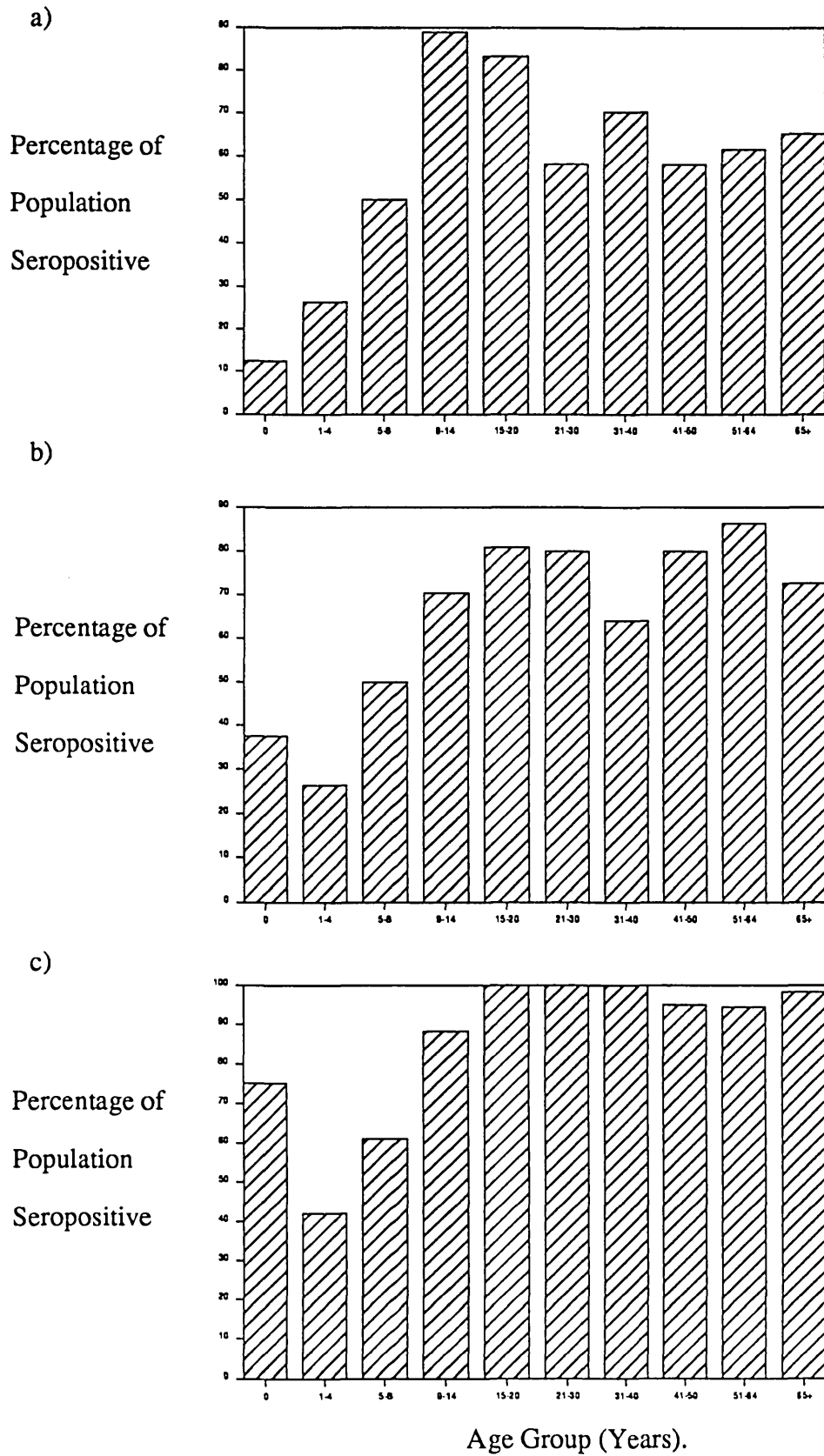
Figure 5.1; Age serological profiles from 1989 showing the percentage of each age group that is positive for a) A/Bel/1/81, b) A/Eng/333/80 and c) A/Hong Kong/68. The cut-off points used to determine whether each sample was positive or negative are detailed in Table 4.2.

In the older age groups of the data for A/Bel/1/81 (see Fig. 5.1(a)) a distinct decrease in the proportions of the populations seropositive can be observed. The peak in the 9 to 20 year age groups is the most striking aspect of this profile, as well as the lack of any significant maternally-derived antibody.

From Fig. 5.1(b) it can be seen that, for the A/Eng/333/80 virus strain, there is a higher prevalence of antibodies in infants aged between 0-5 months, this may be due to maternally derived antibodies. Unfortunately, due to small sample size of the 0-1 year age group, it is not possible to assess whether there is a significant decay in this passively acquired antibody, which would indicate the presence of maternally derived antibodies directed against this strain.

The age-serological profile of A/Hong Kong/68 (Fig. 5.1(c)) follows the pattern characteristic of a virus with a high transmission potential that is endemic in the study population. The proportion of the population seropositive during the young age groups rises sharply with age, giving rise to a high proportion of the older age classes being seropositive. In addition to there is the presence of a high level of possibly maternally derived antibody. The increasing levels of seropositivity seen in older age classes must be attributed to active acquisition of antibody through exposure to the viral strain. It can be seen that from 1 year to 15 years old the level of the population positive to the virus rises from 25% to 80%. After the initial rise the proportion positive stays relatively constant at between 80% and 90% through all older age classes.

Persistently high levels of seropositivity from the 10-15 year age groups upward suggest maintenance through time of continually high rates of viral transmission. This is indicative of an endemic virus (see also the longitudinal trends detailed in section 5.5). A slight decline in seropositivity occurs in middle-age which may be the result of the epidemic cycles of the virus or may simply be a sampling artefact.

Figure 5.1(c) shows the typical age-serological profile of an endemic virus, with high levels of seropositivity in the infant class (<1 year, due to maternally derived antibody), rising prevalence of antibody through 1 to 14 year old children, followed by a plateau in the adult age classes.

a)

Mean

Antibody

Concentration

(log-standardised)

b)

Mean

Antibody

Concentration

(log-standardised)

c)

Mean

Antibody

Concentration

(log-standardised)

Age Group (Years)
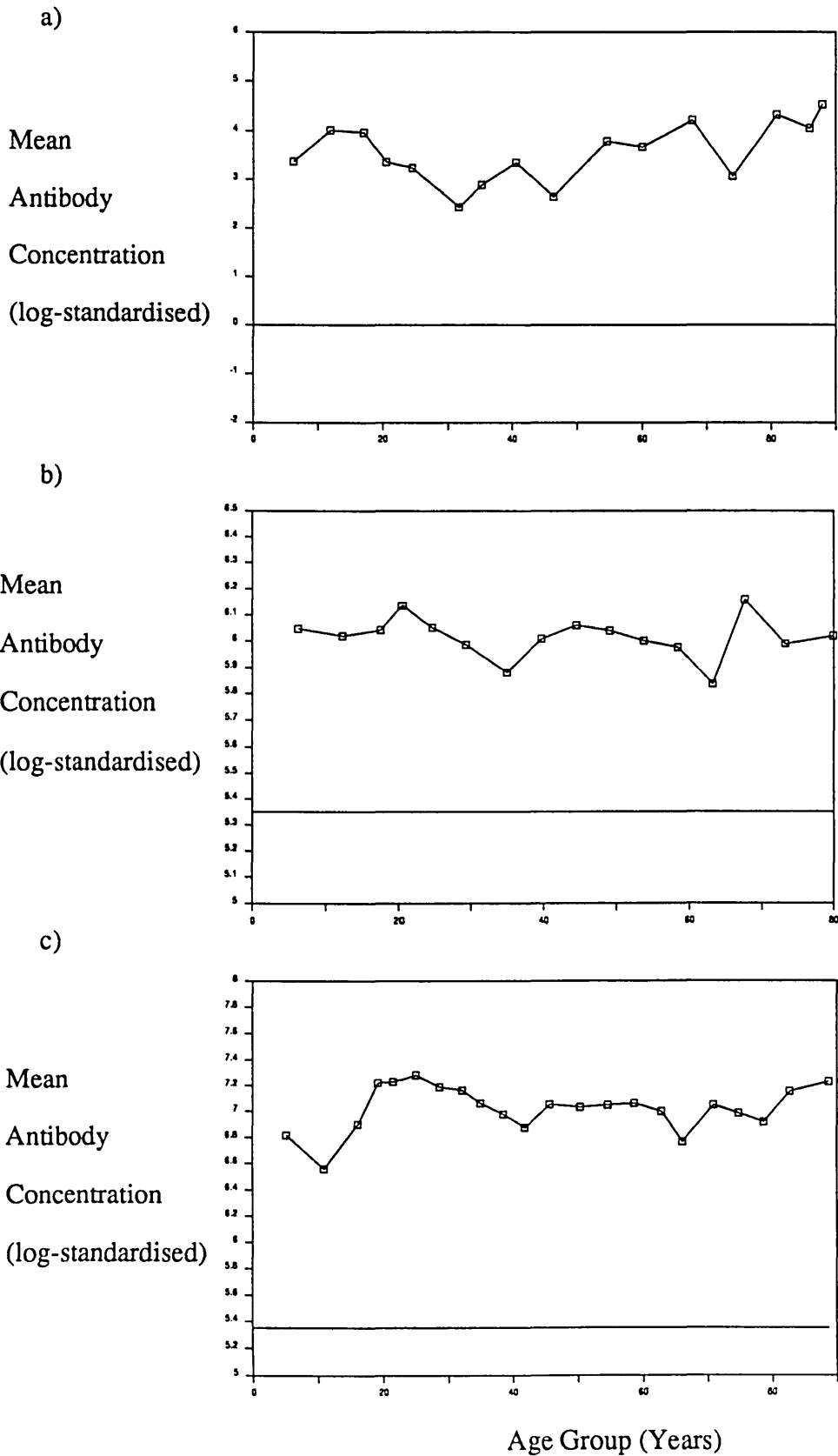
Figure 5.2; Mean antibody concentrations (log-standardised) against age for seropositive individuals, screened against a) A/Bel/1/81, b) A/Eng/333/80 and c) A/Hong Kong/68. Each point represents 25 samples, with the exception of the highest age recorded, where sample sizes are smaller.

The levels of antibodies observed in the sera collected in 1989 indicate that at that time both the A/Hong Kong/68 (H3N2) strain and the A/Eng/333/80 (H1N1) strain were essentially endemic. The A/Bel/1/81 (H3N2) strain, however, appears not to have reached an endemic equilibrium by that point in time. Repeated cross-sectional studies, in which the serological results from different cohorts of individuals screened at regular periods are considered, are detailed in section 5.5. In that section the situation in terms of the endemicity (or otherwise) of these three subtypes of influenza A is discussed.

5.3.2. Age-related trends in specific antibody concentration.

It is possible to use the results of the sera screening to study any trends with age in the mean concentration of actively acquired antibody. This may aid in the interpretation of age-serological profiles. Figure 5.2(a), (b) and (c) show the mean concentration of antibody in the sera of seropositive individuals, collected in 1989, against age, for the three strains under consideration. The data starts at age 1 year and is blocked into fixed sample sizes. The regression analysis data is shown in Table 5.1. From this it can be seen that the changes in the mean antibody levels through age are negligible. This suggests that there is no age-related decline in the antibody levels in an individual for any of the strains of influenza A examined. The horizontal line represents the cut-off points for the three strains to demonstrate that there is a significant difference between the average positive values (shown in Table 5.1) and the chosen cut-off points (see table 2.2).

5.3.3. Age-dependant Variability in Antibody Levels.

It has been shown that variability in antibody levels to antigens of directly transmitted viruses in populations in developed countries can increase with age (Nokes et al. 1986). If levels fall below the cut-off point this can lead to false negatives. If this occurs the observed proportions seropositive in the older age groups may underestimate the true pattern of exposure.

Figures 5.3(a),(b) and (c) show the variance to mean ratios against age for the strains in question for 1989. For the two strains which co-existed during the 1983 epidemic season it can be seen that any variability in antibody concentration is negligible and certainly falls within acceptable limits. However, it can be seen that there is considerably more variation

a)

Variance

To Mean

Ratio

b)

Variance

To Mean

Ratio

c)

Variance

To Mean

Ratio

Age Group (Years).
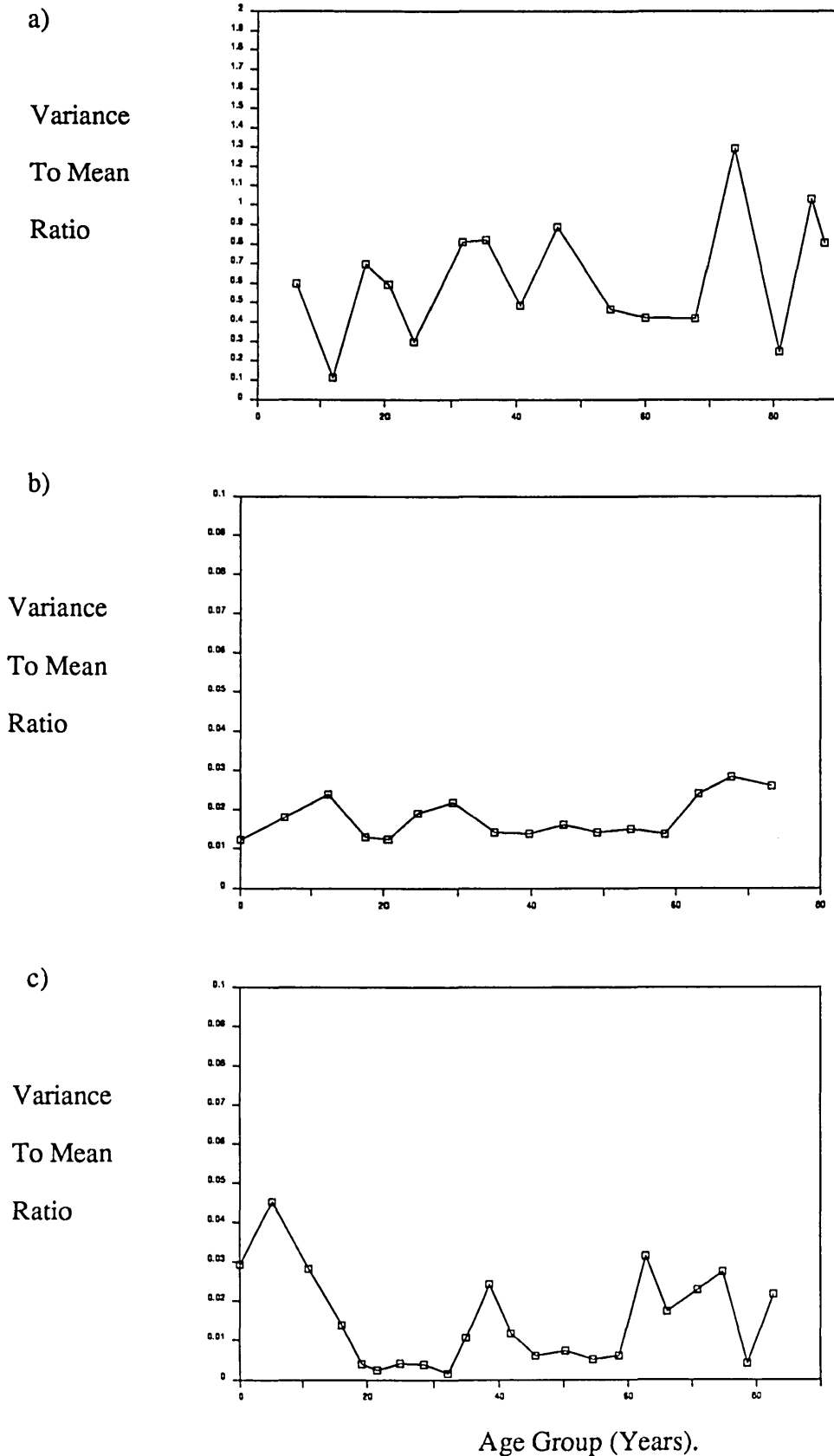
Figure 5.3; The variance to mean ratios for antibody levels (log-standardised values) plotted against age for a) A/Bel/1/81, b) A/Eng/333/80 and c) A/Hong Kong/68. Note that the scale for (a) is an order of magnitude greater than the other two. There is no apparent trend for a change in variability with age for any strain.

Table 5.1. The rate of change in the average antibody concentration through age for the seropositive individuals in the 1989 sera set. Tested against three virus strains.

Strain	Average Conc.*	Rate of Change[+]	r^2 value
A/HK/68	7.026	3.24×10^{-4}	2.55×10^{-4}
A/Eng/333/80	6.015	-5.1×10^{-4}	1.24×10^{-3}
A/Bel/1/81	3.518	8.23×10^{-3}	1.93×10^{-2}

* Log-Standardised Units.

[+] Gradient of Regression line for the correlation of antibody concentration with age. (Significant deviation from the line indicates a trend with respect to age).

for the A/Bel/1/81 strain than for A/Eng/333/80. A/Hong Kong/68 shows a small variation, more comparable to the A/Eng/333/80 strain than the A/Bel/1/81 strain.

5.4. Longitudinal trends.

Horizontal surveys are not ideal for estimating certain epidemiological parameters (Nokes et al. 1989) so it is sometimes useful to consider the change in antibody levels of a population through time (this can be considered as approximating longitudinal surveys on the same cohort of individuals). This can be most easily done by constructing a series of horizontal age-serological profiles that represent the passage of several cohorts of individuals through age and time. Note that samples of a given age class in different years do not consist of the same individuals.

Figures 5.4(a), (b) and (c) show several horizontal age-serological profile's though a course of some 10 years for the three strains of influenza (20 years in the case of A/Hong Kong/68). It is from these figures that the strains are classified as being either endemic or epidemic, depending on the characteristics of the age-serological profile for the years studied. It can, therefore, be concluded that, over the time periods studied, both the A/Hong Kong/68 and the A/Bel/1/81 strains appear to be new arrivals, whereas the A/Eng/333/80 strain appears to be endemic.

a)

1.0
Percentage of
Population
Seropositive

55+ 51-64 41-50 31-40 21-30 15-20 9-14 5-8 1-4 0

1989
1988
1987
1985
1981
1979

Year
of sera
Collection.

b)

1.0
Percentage of
Population
Seropositive

55+ 51-64 41-50 31-40 21-30 15-20 9-14 5-8 1-4 0

1989
1988
1987
1985
1981
1979

Year
of sera
Collection.

c)

1.0
Percentage of
Population
Seropositive

55+ 51-64 41-50 31-40 21-30 15-20 9-14 5-8 1-4 0

1989
1988
1987
1985
1981
1979
1969

Year
of sera
Collection.

Age Group (Years).

Figure 5.4; Horizontal age-serological profiles over a number of years through the period 1969 to 1989 for a) A/Bel/1/81, b) A/Eng/333/80 and c) A/Hong Kong/68. The establishment of the strains can clearly be seen in both (a) and (c), whereas the strain in (b) appears to be endemic for the time period studied.

It is also possible to derive estimates for the force of infection of each virus strain in the community, and its variation through time, from this 'longitudinal' data. These trends in the seropositivity of the sample must be interpreted with some caution, however, since they do not represent actual longitudinal cohort studies. The sera samples used are taken from the same area, and hence population, but they are not the same individuals followed through consecutive time periods.

5.4.1. Strains Endemic in 1989 (A/Eng/333/80 and A/Hong Kong/68).

From Figs. 5.4(b) (showing the longitudinal trends of A/Eng/333/80), 5.4(c) (A/Hong Kong/68) and also Fig 5.5, it can be seen that both these viral strains were at an approximate endemic equilibrium in 1989. It must be stressed that there will be many individuals who will have been alive for a longer period of time than the particular strain has been circulating, and, therefore, the situation observed here is not a true endemic equilibrium of the sort observed with other viruses, such as measles, mumps or rubella. The A/Eng/333/80 strain appears to have been incident before 1979, unfortunately the antigen was exhausted before it was possible to test the 1969 or 1973 sera against this strain to determine the exact year of appearance. It is possible that the antibodies detected here were a result of a previously

Year of sera collection.
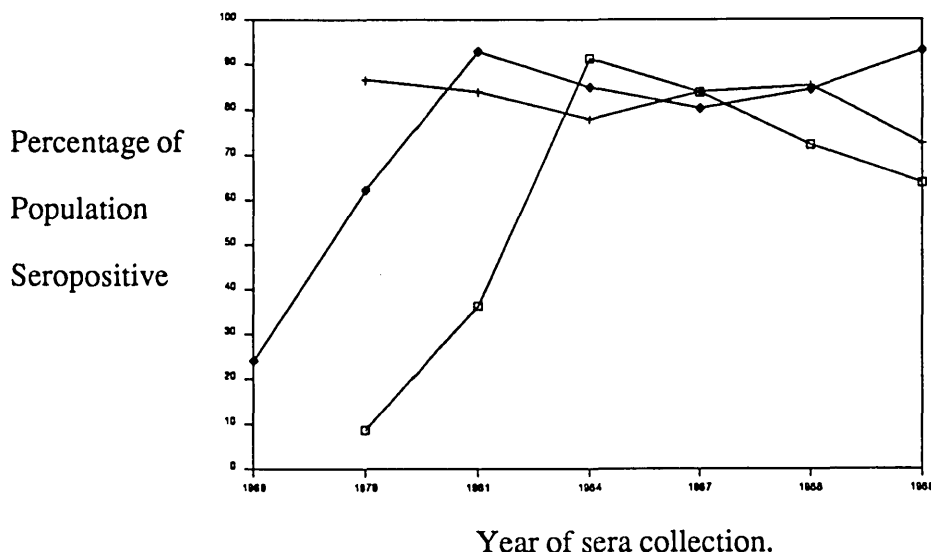
Figure 5.5; The proportion of the population seropositive to the three strains of influenza A through the years 1969 to 1989. The diamonds represent A/Hong Kong/68, the crosses A/Eng/333/80 and the squares A/Bel/1/81. The appearance of the two 'epidemic' strains is obvious, with a small decline in the antibody level through time for two of the strains, but not the third (A/Hong Kong/68).

circulating antigenically similar strain, which would cause the profile to take on this shape. The A/Hong Kong/68 strain, however, can be seen to have been present in the younger age groups at or before 1969. It then spread through the age groups until the situation is reached in 1989, detailed in Fig. 5.1(c), where the profile is the classic shape for that of an endemic virus.

The change in the proportion of the total population seropositive to the strains can be seen in Fig 5.5, where both A/Hong Kong/68 and A/Eng/333/80 are maintained at high levels of seropositivity in 1989, in contrast to the A/Bel/1/81 strain which appears to be declining in prevalence through time. As consequence of these observations it appears likely that the strains A/Hong Kong/68 and A/Eng/333/80 are at an approximately endemic equilibrium (or are approaching this situation) in the 1989 sera set, whereas it appears that the A/Bel/1/81 has not yet reached equilibrium. It is important to consider the age structure of the sera sets used, which may have some bearing on the interpretation of Fig. 5.5. From Fig. 4.1 it can be seen that the distribution of individuals throughout the age groups in all samples is essentially uniform, so all data points presented in Fig. 5.5 can be considered to be equivalent in size and age structure.

5.4.2. Strain Epidemics in 1989 (A/Bel/1/81).

The data records an example of a virus strain (A/Bel/1/81) being introduced into a population of susceptible individuals. In 1979 the only individuals that had apparently been exposed to the virus were a small proportion of the youngest age classes. As the virus continued to circulate it appears that older age groups seroconverted until the situation essentially resembled that of an endemic virus, but without all of the older age groups being 100% seropositive. This may well be indicative of a low force of infection in the middle age groups, which would explain the relatively low levels of prevalence (less than 70%) observed in the 21+ age groups in 1989 (Fig 5.1(a)).

The situation is well summarised by Fig. 5.5 which shows the changes through time of the total proportions of the population seropositive to the three strains. It can be seen that both A/Hong Kong/68 (in 1969) and A/Bel/1/81 (in 1979) induce large proportions of the population to become seropositive, whereas from 1979 onwards all three strains show high levels of seropositivity.

Part II. Age related Variation in the Rate of Transmission.

5.5. Introduction.

The distribution of immunity to an infection which is either epidemic, or considered to be endemic, in a population at one time may be ascertained from age-serological profiles. This was clearly illustrated for various influenza A strains in the first part of this chapter. In general terms the steepness of an age serological profile (ASP) is a measure of the magnitude of transmission of infection in the community; the more rapid the rise, the greater the degree of transmission (Anderson and May (1983b)).

More specifically, the changes with age in the proportion immune, are an indication of the probability of an individual acquiring infection. A quantitative measure of this infection rate is known as the 'force of infection' (denoted by the symbol λ) and may be defined as the per capita rate at which susceptible individuals acquire infection (Anderson (1982), Anderson and May (1982b, 1983a and 1985) and Grenfell and Anderson (1985)). Estimation of the force of infection, λ, is made possible by the use of 'catalytic infection models' which mirror the changes in the proportion immune (i.e the proportion of the population who have experienced infection) recorded by age-prevalence profiles (or alternatively, by prevalence profiles determined from case notification analyses which are discussed in chapter 3). (Muench (1959), Griffiths (1974), Anderson and May (1982b, 1983a and 1985) and Grenfell and Anderson (1985)).

The force of infection, defined above, is not equivalent to the more widely used 'attack rate'. The attack rate is defined as the number of cases in a specified stratification (e.g. age class) of the population over a given time interval, per head of population in that stratification (Grenfell and Anderson (1985)), and not the number of cases per **susceptible** head of population.

Simple observation of heterogeneity in the rate of contact between individuals within and between age classes in a community gives rise to the suspicion that the probability of a susceptible individual becoming infected (in an interval of time) is very much dependant on the age of that individual (e.g. the interactions between school children compared to those between school children and the elderly). Age-dependence in the per capita rate of trans-

mission has been documented for various childhood viral and bacterial infections, including measles, mumps, varicella, pertussis, scarlet fever and rubella (Griffiths (1974), Fine and Clarkson (1982) Anderson and May (1985) Grenfell and Anderson (1985), Nokes et al. (1990) and Cox (1990)). A consistent age-related trend in the force of infection has emerged from these studies: low in young children, higher in older children and adolescents, and low again in adults. One of the aims of this study is to see if this holds true for the various subtypes or strains of influenza A, especially in light of the results from chapter 3, which indicate otherwise.

In the same way that mass immunisation acts to increase the average age at infection (Knox (1980), Fine and Clarkson (1982), Anderson (1982) and Anderson and May (1982b and (1983a)) it appears probable that cross-immunity conferred from one strain to another acts to raise the average age at infection for both strains (this concept is dealt with in detail in chapter 6). For this reason the nature of age-dependant variation in the force of infection may well have a considerable bearing on the acquisition of natural immunity to related strains of influenza A. The risk of serious complications arising from infection is itself age-dependant (Hers et al. (1958), Finland et al. (1945), Martin et al. (1959) and others). Accurate determination of age-related rates of infection should, therefore, be regarded as a prerequisite for research involving the development of models which explore the impact of cross-immunity between strains on disease incidence.

Serological data is the most reliable epidemiological information upon which to base such estimates (see also chapter 3 for case notification data) (Anderson and May (1983b) and (1985)). However, until now, there has been a lack of detailed, finely age-stratified serological data for influenza in the UK. The surveys, described in Part I of this chapter, go some way to rectifying this situation.

The force of infection of any particular strain of a virus is dependent on three things. The infectiousness of the virus strain, the rate of contact between infected and susceptible individuals and the number of infectious individuals in the population at the time. From this it might be supposed that the characteristics of a given strain will be reflected in the pattern of the epidemic that results from the introduction of that strain into the community.

The force of infection can be measured in various ways. It can be measured directly from the serological profile which is taken during the year in which the strain first caused a number of cases (the year of incidence), or from a 'cohort' of individuals (although it must be stressed that this is not a cohort of the same individuals, merely individuals of an equivalent age range for that year), as it ages, through time. By looking at a 'cohort' of individuals who are seropositive to A/Bel/81 or A/Hong Kong/68 through time an estimate can be made of the change in the force of infection as this 'cohort' ages. If the virus is considered to be endemic, the standard catalytic infection model can be applied to those individuals who have been born subsequent to the epidemic, but not to the older age groups. These values can then be compared with those which were obtained by the methods based on serological studies at one point in time.

In the following sections, age-related changes in the rate of influenza transmission are determined from the age-serological profiles for the South Yorkshire district, employing either a direct method, or the catalytic infection model described in the next section, depending on the equilibrium state of the virus strain. The aim is to determine the age dependant nature of the force of infection for various influenza strains, which has only been considered in anecdotal form for individual strains of the influenza virus up to this time (Collins (1929) and Glezen (1982)). The data is also used to provide precise age-dependent estimates of this parameter for a mathematical model of influenza A transmission, which is then used in chapter 6 to explore the interactions between two co-existing strains. A discussion then follows on the extent to which these results are representative of influenza in this country and on the factors which might affect confidence in the authenticity of estimates of infection rates from serological data.

5.6. The Catalytic Infection Model.

Age-specific forces of infection, $\lambda_{(a)}$, can be estimated from the age-stratified serological data for the South Yorkshire area detailed in chapter 4, using the method described by Anderson and May (1983b). In their approach, the rate of change, with age, in the proportion of the population immune to an infection by age a ($F_{(a)}$) is represented by a catalytic infection model which takes the form;

$$dF_{(a)}/da = \lambda_{(a)}[1-F_{(a)}] \tag{5.1}$$

This differential equation has the solution

$$F_{(a)} = 1 - \exp[1 - \int_0^a \lambda(x)\, dx] \tag{5.2}$$

Previous models of this nature assume the force of infection, λ, to be age independent (Muench (1959)) or to increase linearly with age (Griffiths (1974) and Anderson and May (1982b)). Anderson and May (1985), however, represent the force of infection as a function of age a. If $x = (1-F)$ then from equation (2)

$$\lambda_{(a+1/2\,\Delta a)} = -\ln. \frac{x_{(a+\Delta a)}}{x_{(a)}} \Big/ \Delta a \tag{5.3}$$

More complex methods have been developed, which are discussed later, but for this study $\lambda_{(a)}$ is estimated from observed changes with age in the proportion immune, (taken directly from age prevalence profiles) using equation (5.3). This method was chosen in place of any other possible methods (e.g. the polynomial approximation method described by Grenfell and Anderson (1983b)) because only 5 data points were available for use once the individuals had been clumped to form significant numbers within any one age group.

The accuracy of this method in the determination of age-related forces of infection will be affected by the validity of the assumptions inherent in the model. These assumptions are (Grenfell and Anderson (1985), Anderson and May (1985)) as follows;

(a) the force of infection is constant with time (or at least exhibits constant recurrent cycles around an average equilibrium level);

(b) the infection induces lifelong immunity to reinfection by the same strain of influenza and does not increase the mortality of those infected;

(c) horizontal age-related changes in the proportion of the population who have experienced infection mirror longitudinal changes for a cohort of individuals.

(d) there is no age-dependant bias in reporting;

(e) the case notifications are of infection with the virus, and not only of clinical cases of disease.

For a stable population, with the virus at an endemic equilibrium, these assumptions are roughly satisfied. However, in the case of the A/Bel/81 strain, these assumptions obviously do not hold true (Fig 5.4(a)), so a direct method must be employed for the year in which the virus was incident. When estimating the age-dependent force of infection in this manner it is assumed that the proportion of the population seropositive is directly proportional to the

force of infection in that year. This is easily explained by considering equation (5.3) and assuming that the proportion susceptible at age (or time) 0 will be 1.0. That is, in the year before the virus appeared, no individuals were seropositive, but in the year under consideration a proportion of the population became seropositive in direct relation to the size of the force of infection acting upon a specific age group.

5.7. Force of Infection Estimates.

5.7.1. Using the Catalytic Infection Model.

Using the catalytic infection model described it was possible to estimate the forces of infection for the two strains under consideration; A/Hong Kong/68 and A/Eng/1/81. Table 5.2 shows the estimated forces of infection values for the various age classes.

These estimates were made from the 1989 data set where the strains are assumed to be approaching an equilibrium state. Under this assumption the estimated forces of infection are only determined for those age groups for which the force of infection value can be considered over the time period in question. Thus, only those age classes which could have been exposed to the virus strains (i.e. those ages which are less than the time for which the strain in question has been circulating) have been considered in this analysis. These ages are less than 20 years old for the A/Hong Kong/68 strain, and less than 10 years for the A/Eng/81 strain.

From Table 5.2 it can be seen that the age-specific forces of infection for the A/Hong Kong/68 strain of influenza appear to be highest in the 15-20 year age group. There are not many conclusions which can be drawn about the pattern of transmission with respect to age from these values, since it is not possible to estimate any forces of infection for the older age groups which have not been exposed to the virus strain for the whole of their lives. However, the values can be used in the estimation of the forces of infection for the model which is described in the following chapter.

5.7.2. Direct Estimation of the Force of Infection.

From direct measurement of the proportion of the population which expresses antibody to a specific virus strain which is acting on a 'virgin' or naive population (i.e. highly susceptible) it is possible to determine the age-dependant forces of infection for a particular

a)

Force of
Infection.
(year^{-1})

Age Group.

b)

Force of
Infection
(year^{-1})

Age Group
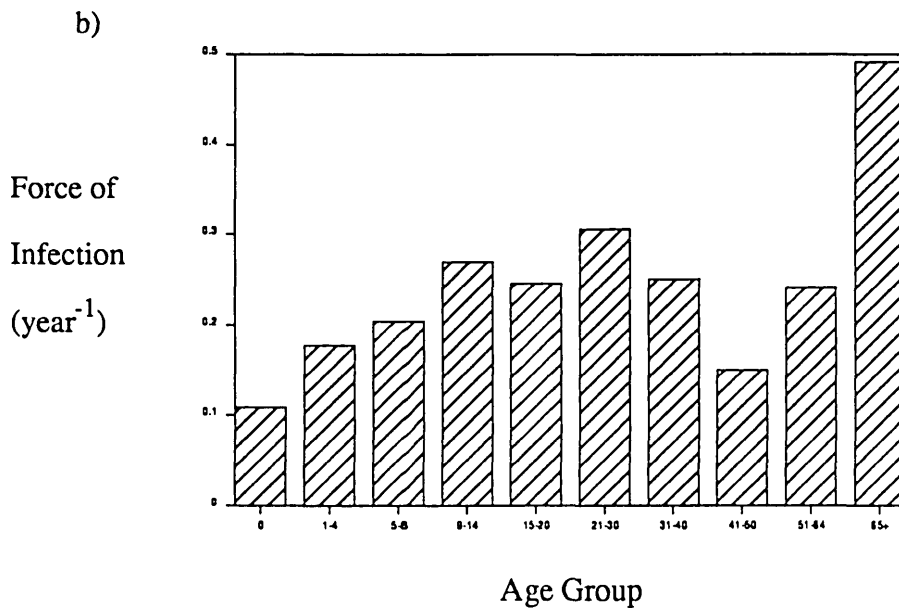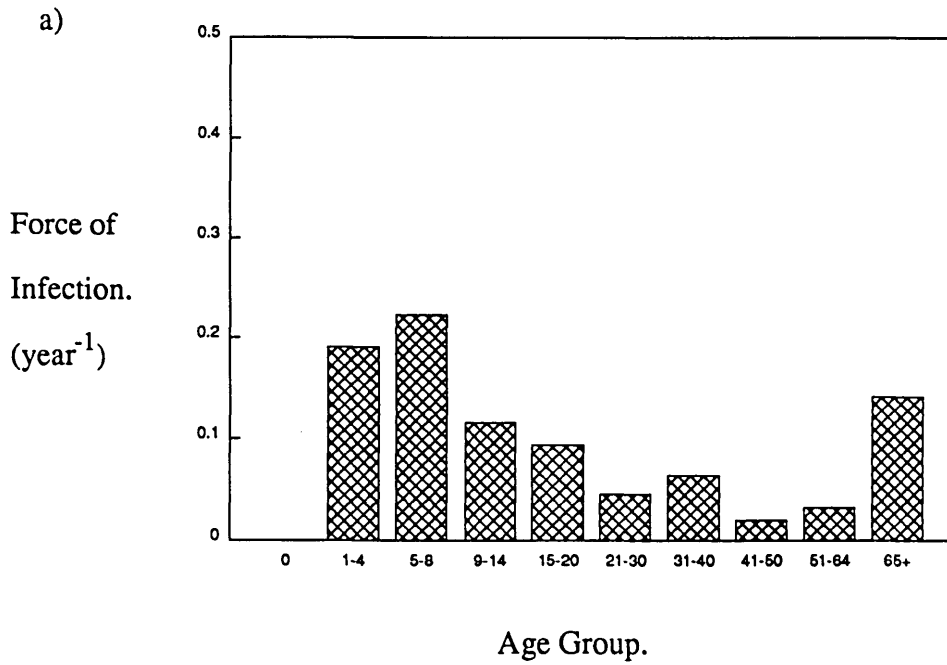
Figure 5.6; The estimated forces of infection from the proportion seropositive a) in 1979 for A/Bel/1/81 and b) in 1969 for A/Hong Kong/68. The method of estimation is from direct measurement of the proportion of the population immune to the strain of virus. As in Fig. 5.7 the force of infection for the A/Hong Kong/68 strain has maximum values at a greater age than for A/Bel/1/81.

strain in that season. This has been done for the two strains which can be seen to be incident in a specific year, namely A/Hong Kong/68 and A/Bel/1/81.

This method for the estimation of age-specific forces of infection gives the most information. It is possible to compare the pattern of the age specific forces of infection for A/Hong Kong/68 (Fig. 5.6(b)) with the patterns of infection found during the analysis of the case notification data (Fig. 3.6). Both patterns of infection show a peak around 20 years of age, followed by a high level of 'infectivity' in the 65+ age group. This indicates a similar situation with respect to the patterns of infection; it is probable that more of the sera samples collected from the oldest age group were referred to General Practitioners as a result of some influenza-linked respiratory disorder, thus leading to over-reporting of cases (in terms of real infection) in this age group.

There is a different pattern observed in the case of the A/Bel/1/81 strain (Fig 5.6(b)), which shows a higher force of infection in younger individuals, followed by a gradual increase through age. Those estimates for the A/Hong Kong/68 strain (Fig. 5.6(a)) agree well with the estimates from the endemic situation (Table 5.2).

5.7.3. Estimation of Force of Infection from Longitudinal Study.

In the same way that force of infection estimates could be determined for each age group in section 5.6, it is possible to calculate the change in the force of infection through time from the longitudinal data.

Using the catalytic infection model described in section 5.6 it is also possible to estimate age dependant forces of infection from this data. By following a 'cohort' of individuals (as described earlier) through age and hence time, it is possible to construct an age serological profile which takes into account the change of the age-dependent forces of infection through time (Fig 5.9). From this figure it can be seen that there is a distinct difference between the age groups <1 year and 5-8 years for both the A/Hong Kong/68 strain and the A/Bel/81 strain. However, this difference remains essentially the same through time, although the values of both age dependent forces of infection change through time.

The force of infection estimates from the longitudinal study are $0.076 \, \text{year}^{-1}$ for A/Hong Kong/68 and $0.164 \, \text{year}^{-1}$ for A/Bel/1/81. As both viruses approach an endemic equilibrium (1981 for A/Hong Kong/68 and 1985 for A/Bel/1/81) the average force of infection is ap-

a)

Proportion of

Age Group

Seropositive.

0.48

0.27

0.33

0.17

Year sera Collected.

b)

Proportion of

Age Group

Seropositive.

0.36

0.25

0.57

0.14

Year sera Collected.
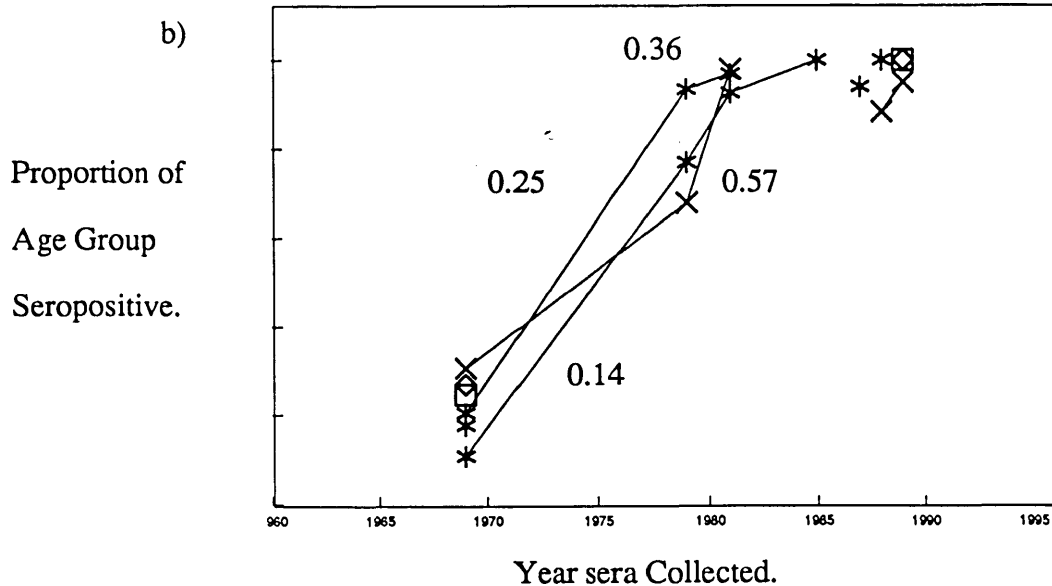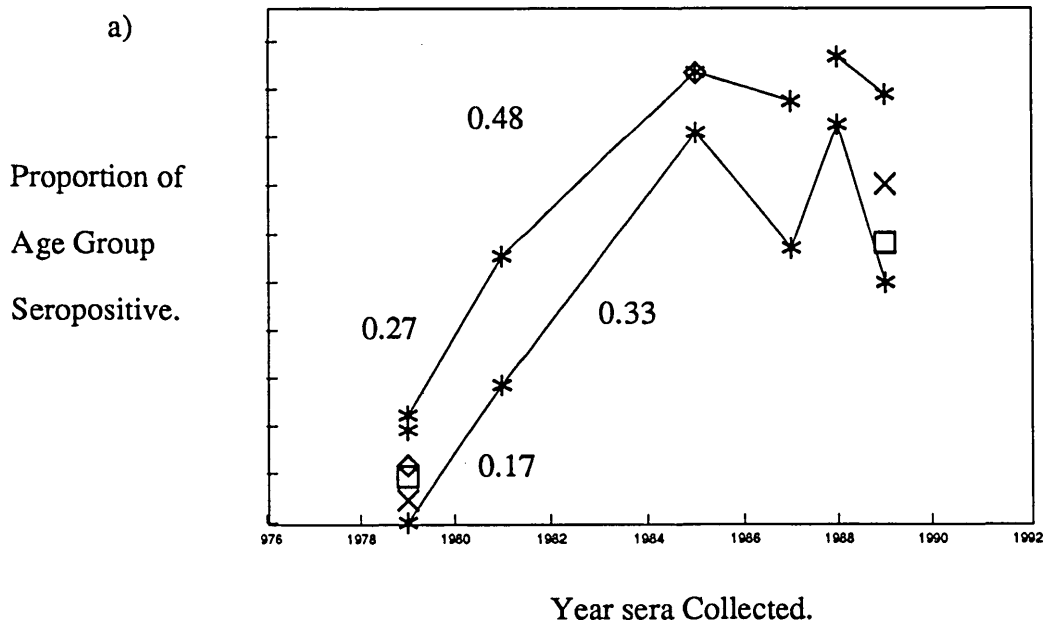
Figure 5.7; The change in the proportion seropositive of selected age groups which are followed through time and, hence age, across the series of horizontal age-serological profiles shown in Fig. 5.4 for a) A/Bel/1/81 and b) A/HK/68 The numbers adjacent to the lines representing the changes in proportion seropositive are the age- and time-dependent forces of infection for each 'cohort' during each time period defined per year. The forces of infection were determined using equation (5.3). The upper of the two starred lines represents the age group which was 5-8 years old in the first year of sera collection. The lower of the two starred lines is for infants <1 year old in the same year. In graph (b) the line shown with a cross is the 9-14 year old group (no FOI's are shown for this group).

Table 5.2. Estimates of the age-dependant forces of infection (FOI) from all sources of serological data. Estimates were made utilising equation (5.3) from the text.

Strain of Influenza	Age	FOI (year^{-1}) A	FOI (year^{-1}) B	R_0 *
A/HK/68	<1	0.0	0.108	19.8
	1-4	0.182	0.177	
	5-9	0.099	0.204	
	10-14	0.299	0.269	
	15-44	0.323	0.265	
	45-64	-	0.200	
	65+	-	0.491	
A/Eng/333/80	<1	0.0	-	8.7
	1-4	0.102	-	
	5-9	0.097	-	
A/Bel/1/81	<1	-	0.0	8.5
	1-4	-	0.190	
	5-9	-	0.222	
	10-14	-	0.115	
	15-44	-	0.064	
	45-64	-	0.025	
	65+	-	0.141	

A = virus strain at approximate equilibrium in the population.

B = virus strain incident in that year (1969 for A/HK/68 and 1979 for A/Bel/1/81).

* Estimated from equation 6.18, using average forces of infection from this table.
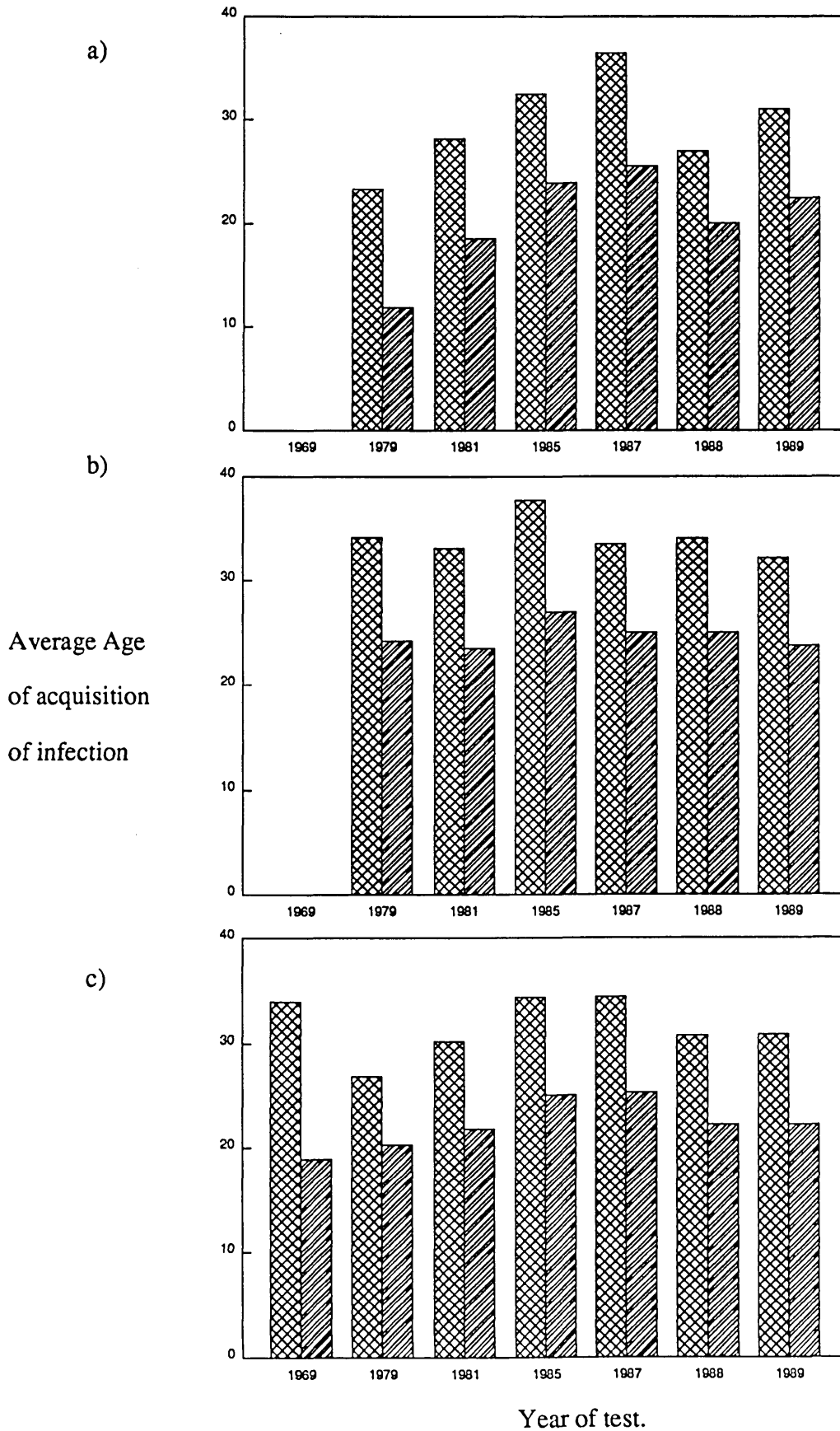
Figure 5.8; The changes in the average age of infection for the three strains a) A/Bel/1/81,
b) A/Eng/333/80 and c) A/Hong Kong/68 over the years of the study. Two average ages
are shown; the first is calculated, as described in the text, using the 65+ age group (the
cross-hatched bars); and the second ignores this age group (diagonally hatched bars).
The actual value probably lies somewhere between the two.

proximately 0.2 year^{-1}. This value is in general agreement with the age-dependant forces of infection listed in Table 5.2.

It is, therefore, possible to conclude that the age-dependent forces of infection are increasing continuously after the initial year of incidence for each strain.

A summary of the estimates of the forces of infection is given in table 5.2.

5.8. Average Age at First Infection.

By considering the serological profiles for the years in which the strains (where applicable) were incident, it is possible to determine the average age at which individuals became seropositive. This can be done simply by calculating the weighted mean age of seroconversion for a specific year.

If this is done for the years following the appearance of the virus strain, it is possible to get an indication of the transmission dynamics of the strain in terms of age classes. From Fig 5.10 it can be seen that the average age of seroconversion remains relatively constant through time for both A/Eng/333/80 and A/Hong Kong/68, but appears to be rising over time in the case of A/Bel/1/81. It is important to note, in terms of the actual age of infection (or seroconversion), that the inclusion, or not, of the oldest age group (65+ years old) considerably affects the magnitude of this value. Since there is reason to believe that there is some over reporting in this age group (as discussed earlier) values including, and ignoring, this age group are given in Table 5.2. It is assumed that the actual value lies somewhere between the two.

5.9. Evidence of Cross-Immunity between Strains.

5.9.1. Introduction.

Some mention has been made of the potential cross-reactivity of some of the antibodies present in the serum of the individuals tested. To determine whether any relationship is present the data are considered in terms of exposure to combinations of antigenic strains of influenza A. As mentioned previously, because of the possible element of cross reactivity in the test for the presence of antibody specific to one strain, the presence of antibody does not necessarily indicate previous exposure to infection. Some individuals, therefore, will not

have been infected with a subsequent strain as a consequence of possessing protective antibodies to an earlier strain. However, it is possible to measure the number of individuals with antibody directed against more than one strain.

Individuals who appear to be positive to more than one strain could possess these antibodies for any one of three reasons. Infection with one strain could produce antibodies which are cross reactive with another strain, antibody generated by an early strain might confer immunity to the second strain, and thus be detected in the test, or an individual could have been infected by both strains consecutively. Obviously, the first two scenarios may be in fact the same, and it is this possibility that requires further analysis.

One hypothesis to test whether 'double-positives' occur due to cross reactivity or through the effect of cross immunity is as follows. By determining the number of individuals that could be expected to be positive to two strains (have been infected with both strains), on the assumption that no cross-immunity occurs, it is possible to compare the observed results from serological analysis with these expected results. If the observed number of double-positives equals that of the expected number, it can be assumed that these double positives are indeed due to infection with two strains. If, however, the number of observed double-positive individuals is greater than the expected number, this is indicative of the antibody being present in more individuals than have actually been infected with both strains of the virus. This situation indicates that cross reactivity is occurring in the serological screening procedure. If the observed number of double positive individuals is less than the expected number, it can be assumed that there are less individuals with antibody to both strains than would have been expected to be exposed to both strains. Thus, the presence of antibody to an early strain has prevented infection with a second, antigenically similar strain. This implies that the same antibodies are causing immunity against both virus strains.

In this method of determination, two factors must be taken into account. First, the results rely heavily on the reliability of the calculated expected number of double-positive individuals. Also, there is a strong possibility that antibodies which are cross reactive are also protective against challenge from either of the strains for which they give a seropositive result.

5.9.2. Method.

If all three strains of influenza virus which are under study are considered, there are three possible two-way interactions. Individuals can be double-positive to A/Eng/333/80 and A/Hong Kong/68; A/Eng/333/80 and A/Bel/1/81; or A/Hong Kong/68 and A/Bel/1/81.

For ease of analysis the data are displayed in the form of three two-way tables for each year (Tables 5.3 (a), (b) and (c) show the data for 1989). From two-way tables of this sort the expected frequencies for each cell of the table can be calculated (based on the null hypothesis of independance) and compared with the observed frequencies. To compute the expected frequencies for a cell in any two-way table, the sum of the row which contains the cell is multiplied by the sum of the column, and the product is divided by the overall total. It must be noted that any test of independance between the expected and observed values can only test whether or not these values are manifested independantly, and not whether they occur in any given proportion.

By constructing three two-way tables for the various scenarios it is possible to determine values for the goodness of fit of the observed cell frequencies to their expectations. These

Table 5.3. Two-way tables for the year 1989 for all strains showing observed frequencies, sums of columns and rows, and the sum of the chi-squares for each table. All other years of the study demonstrated similar patterns, as shown in Table 5.4.

	HK+	HK-		
Bel +	288	3	291	$\Sigma\chi^2=43.0$
Bel -	140	29	169	
	428	32	460	

	HK+	HK-		
Eng+	325	10	335	$\Sigma\chi^2=30.0$
Eng-	103	22	125	
	428	32	460	

	Eng+	Eng-		
Bel+	232	59	291	$\Sigma\chi^2=19.1$
Bel-	103	66	169	
	335	125	460	

N.B. There are 21 individuals who are negative to all three strains.

Table 5.4 Sum of Chi-squares for years 1985, 1987, 1988 and 1989, for all strains of the influenza A virus tested.

Year	Strains	Chi-squared value
1989	Bel vs. HK	43.0
	Eng vs. HK	30.0
	Bel vs. Eng	19.1
1988	Bel vs. HK	48.3
	Eng vs. HK	24.3
	Bel vs. Eng	14.5
1987	Bel vs. HK	52.1
	Eng vs. HK	28.1
	Bel vs. Eng	27.3
1985	Bel vs. HK	75.2
	Eng vs. HK	68.4
	Bel vs. Eng	60.3

values are determined assuming a null hypothesis of no cross-immunity between strains and the chi-square test of independance.

5.9.3. Results.

From Table 5.3 it can be seen that there is a significant difference between the observed and expected values for all three strains tested for in 1989. The data shows that in each case there were greater numbers of observed double-positive individuals than expected, which indicates evidence of cross reactivity in the diagnostic test. It can be see from Table 5.3 that the cells showing most discrepancy are those for the double-negative individuals. This is due to there being less individuals in these cells than expected, which confirms the theory indicating cross-reactivity in the serological test. These values are shown for the 1989 data set, but all other years showed a similar pattern. However, before these analyses are interpreted as suggesting that the ELISA tests are of no value, it must be remembered that these cross-reactive antibodies could, themselves, lead to a protective response. It is interesting to

note that after 1981 (the year of introduction for A/Bel/1/81) the Chi squared values always retain the same ranking (see Table 5.4), with the A/Hong Kong/68 and A/Bel/1/81 strain value greater than the other two. Both these strains are of the H3N2 subtype, whereas A/Eng/333/80 is H1N1. This indicates that whatever reason is behind the cross reactivity, it is consistently stronger for A/Bel/1/81 interacting with A/Hong Kong/68 than for any other combination.

5.10. Discussion.

Age-serological profiles constructed from horizontal, cross-sectional serological data, have depicted the distribution of immunity to three separate strains of influenza A in the South Yorkshire area. The differences in the rate of acquisition are clear and to a large extent reflect the relative transmission potentials and, therefore, the innate infectiousness of the three strains.

The rapid increase in seropositivity with age, and the maintenance of high levels of immunity throughout the adult age classes seen for the strains, suggests that after initial epidemics, the strains are endemic within the community. The patterns in the age-serological profiles are similar to those seen for the common childhood diseases (mumps and measles) in developed and developing countries (Nokes et al. (1989) Cox (1990) for mumps, and Anderson and May (1983b) and (1985) and McLean and Anderson (1988) for measles). The patterns seen for Bel/1/81 indicate that the virus was first epidemic in the early part of 1980 and remained circulating in the population thereafter. These same patterns are evident for the A/Hong Kong/68 strain from 1969 onwards. The variability, with age, in levels of immunity for the A/Eng/333/80 strain suggests a semi-cyclic temporal change in the rate of transmission of the virus (ie recurrent or sporadic epidemics). It is useful to note, at this stage, that both the A/Hong Kong/68 and A/Eng/333/80 strains are of the H1N1 subtype, whereas the A/Bel/1/81 strain is of the H3H2 subtype. The ramifications of this are discussed in detail in chapter 7.

The apparent endemicity of the strains after initial occurrence is very surprising, considering that no record of illness during the off-season months is recorded (Glezen,W.P. (1980)), and that after the initial epidemics, no significant number of case notifications are recorded (see chapter 3). One explanation is that the results observed reflect a lack of spe-

cificity in the antigen (described in chapter 4). However, this explanation does not account for the epidemic appearance of the A/Bel/1/81 strain, nor does it seem likely in the light of the purification process that was employed to obtain the antigen.

The likely transmission dynamics of each virus strain within the population has been used to help explain observed trends with age in mean specific antibody concentrations. High rates of re-exposure to the viruses, particularly in the younger age classes (circa 15 years old) but most likely throughout all age classes, may explain why no decay in mean antibody levels was observed as individuals age. These stable mean levels of antibody seen throughout the age classes are unlike those seen for rubella (Nokes et al. (1986)), and may well result from regular re-exposure, since no lessening of the infection in the younger age classes is observed (which indicates significant levels of the virus in the community). The recurring epidemic character of the A/Eng/333/80 strain in particular complicates the interpretation of age-related changes in antibody concentrations; during an epidemic a proportion of all ages may be infected over a short interval of time.

Using the catalytic infection model it was possible to estimate the forces of infection for the two strains considered to be approaching equilibrium, A/Hong Kong/68 and A/Eng/1/81, from the 1989 data set. Under this assumption the estimated forces of infection are only determined for those age groups for which the force of infection value can be considered over the time period in question. The age-specific forces of infection for the A/Hong Kong/68 strain of influenza appear to be highest in the 15-20 year age group, although there are not many conclusions which can be drawn about the pattern of transmission with respect to age from these values, since it is not possible to estimate any forces of infection for the older age groups which have not been exposed to the virus strain for the whole of their lives.

From direct measurement of the proportion of the population which expresses antibody to a specific virus strain which is acting on a naive population it is possible to determine the age-dependant forces of infection for a particular strain in that season. This has been done for the strains A/Hong Kong/68 and A/Bel/1/81; it is possible to compare the pattern of the age specific forces of infection for A/Hong Kong/68 (Fig. 5.6(b)) with the patterns of infection found during the analysis of the case notification data (Fig. 3.6). Both patterns of infection show a peak around 20 years of age, followed by a high level of 'infectivity' in the 65+ age group. This apparent rise in the transmission rate may be due to an increase in the pro-

portion of this age group which is susceptible to infection (possibly caused by a gradual loss of immunity through time and hence age). However, the same effect can occur due to a bias in reporting in this age group, leading to an excess of cases being reported compared to the number of individuals in the group. It is probably a combination of both effects which leads to the observed pattern. There is a different pattern observed in the case of the A/Bel/1/81 strain (Fig 5.6(b)), which shows a higher force of infection in younger individuals, followed by a gradual increase through age. Estimates for the A/Hong Kong/68 strain (Fig. 5.6(a)) agree well with the estimates from the endemic situation (Table 5.2).

The two strains for which the change in incidence can be followed by a series of cross sections from the population are A/Hong Kong/68 and A/Bel/1/81. It can be seen that there is a distinct difference between the age groups <1 year and 5-8 years for both the A/Hong Kong/68 strain and the A/Bel/81 strain. However, this difference remains essentially the same through time, although the values of both age dependent forces of infection change through time. It is, therefore, possible to conclude that the force of infection increases continuously after the initial year of incidence for each strain in all the age classes that were analysed.

Thus, force of infection estimates obtained from the various approaches described in this chapter demonstrate age-dependency in transmission rates. The high estimate for the 15-20 year old age group can be contrasted with the lower estimates in the young, which suggests that the rates of mixing of individuals within age groups is far from homogeneous.

An essential aspect of further studies of this sort must be the acquisition of sera sets with finer age-stratifications in the younger age groups (most notably for the 0-10 year old range). This would enable a more thorough analysis to be performed, with the potential to determine precisely the changes in the force of infection in a population through age (as determined from age-stratified serology). Essential data can be gathered by following a cohort of individuals, with respect to their acquisition of immunity against specific virus strains, through the course of their lives. It is then possible to determine exact changes in the force of infection of a virus, in an individual, through time. Data of this sort gives a more accurate representation of the acquisition of immunity in a population than several consecutive studies performed on different cohorts of individuals, as detailed here.

The average ages of infection for the various strains (estimated from the proportion of each age group seropositive to each strain) is consistent with those recorded in other studies (see Table 3.1).From Fig 5.10 it can be seen that the average age of seroconversion remains relatively constant for both A/Eng/333/80 and A/Hong Kong/68, but is rising through time in the case of A/Bel/1/81. In all cases the average age of seroconversion lies between 20 and 30.

There is a significant difference between the observed and expected values for all three strains tested for cross reactivity, where the expected values are calculated assuming that there is no cross-immunity between strains of the same virus. In each case there were greater numbers of observed double-positive individuals than expected, which provides evidence of cross reactivity in the serological test employed. These values were recorded from the 1989 data set, and all other years showed a similar trend. It must be remembered that these cross-reactive antibodies could, themselves, lead to a protective response. Whatever reason is behind the cross reactivity, it is consistently stronger for A/Bel/1/81 interacting with A/Hong Kong/68 than with any other combination. As has been discussed in chapter 4, considerable precautions were taken to avoid cross-reactivity occurring in the serological test, with purification of the antigen and the exhaustive optimisation of the ELISA procedure. However, it is possible that the results observed here are due to cross-reactivity occurring, and are thus not entirely indicative of a true result, although this in turn might indicate cross-immunity. The analysis of these results do, however, rule out the possibility that infection with one strain might lead to an individual being more susceptible to infection with a second, similar strain, and thus being in some way predisposed to infection from other similar strains of the same virus.

Values for the basic reproductive rate can be estimated from the data presented in this chapter. It was shown in Table 5.2 that whereas two strains of influenza, A/Eng/333/80 and A/Bel/1/81, had Ro values of between 8 and 9, the A/Hong Kong/68 strain had a value of approximately 20. Although no explanation can be offered for this disparity in magnitude between the strains, it is interesting to note that the A/Hong Kong/68 strain appears to have been circulating for longer than the other two by some 10 years. The long term persistence of this strain may be a consequence of its higher reproductive potential.

For infection with the influenza virus, as with predominantly childhood viral infections, it has long been considered that the degree of mixing between individuals, within and between age classes, plays a crucial role in the net rate of transmission of each strain within the population (Glezen (1980) and Anderson and May (1985)). The results from both the case notification studies in chapter 3 and those from the serological investigation conducted here show a significant age-dependant variation in the rate of transmission of the virus. As a consequence of this the model used in chapter 6 represents an age-structured population.

Chapter Six: A Model with Heterogenous mixing with respect to age.

6.1. The Model.

In this chapter the model described in chapter 2 is considered with age-structuring of the population. This is done by extending the model to include an age-related component, thus producing a set of partial differential equations which describe the flow of individuals between the various classes with respect to time and age. This is done to allow the observed heterogeneity in the forces of infection between age groups to be investigated in a numerical manner. In this way it should be possible to determine whether the general patterns of infection observed are reflected by the numerical analysis of this model.

6.1.1. Introduction to the Partial Differential Equation Model.

The following are the partial differential equations describing the rates at which individuals move from one class to another.

$$\delta X/\delta a + \delta X/\delta t = -(\mu(a) + \lambda_1(a,t) + \lambda_2(a,t))X(a,t) \qquad (6.1)$$

$$\delta Y_i/\delta a + \delta Y_i/\delta t = \lambda_i(a,t)X(a,t) - (\mu(a) + \gamma_i)Y_i(a,t) \qquad (6.1)$$

$$\delta Z_i/\delta a + \delta Z_i/\delta t = (1-\sigma)\gamma_i Y_i(a,t) - (\mu(a) + \lambda_j(a,t))Z_i(a,t) \qquad (6.3)$$

$$\delta V_i/\delta a + \delta V_i/\delta t = \lambda_i(a,t)Z_j(a,t) - (\mu(a) + \gamma_i)V_i(a,t) \qquad (6.4)$$

$$\delta W/\delta a + \delta W/\delta t = \sigma\gamma_1 Y_1(a,t) + \sigma\gamma_2 Y_2(a,t) + \gamma_1 V_1(a,t) + \gamma_2 V_2(a,t) - \mu(a)W(a,t) \qquad (6.5)$$

The total population size, N, is expressed thus;

$$N(a,t) = X(a,t) + Y_1(a,t) + Z_1(a,t) + V_1(a,t) + Y_2(a,t) + Z_2(a,t) + V_2(a,t) + W(a,t)$$

The dynamics of the total population are:

$$\delta N/\delta a + \delta N/\delta t = -\mu(a)N(a,t)$$

The boundary conditions for X, Y_i, Z_i, V_i and W are as follows;

$$X(0,t) = \int_0^{\omega} \mu(a)N(a,t)\,da$$

$$Y_{i(0,t)} = Z_{i(0,t)} = V_{i(0,t)} = W_{(0,t)} = 0$$

That is to say that all newborns are susceptible to infection from either strain (1 or 2). Throughout the above equations $i = 1$ or 2, where j is the opposite (i.e. 2 or 1) and the value for σ lies between 0 and 1. In addition, the initial conditions for X(a,t) etc. are assumed to be known for time t_0 (i.e. the starting age distribution).

The compartmental diagram for the system is shown in Fig. 2.2, which is equivalent to the non age-structured model described in chapter 2.

6.1.2. Parameters Involved in the Model.

(a) The Proportionality Constant.

The function $\beta_i(a,a')$ is exactly analogous to the constant β_i described in section 2.2.2.a, with the exception that it is dependant on the ages of both the susceptible and infectious individuals. Therefore $\beta_i(a,a')$ is determined by the degree of contact between susceptible individuals of age a and infectious individuals of age a', and the likelihood of a susceptible individual acquiring infection from an infectious individual.

As with many similar studies (Anderson and May (1984) for example) the constant of proportionality is incorporated into the model by dividing a lifetime into a number of discrete age classes and assuming that for susceptible individuals in the kth age class, and infectious individuals in the mth age class, $\beta_i(a,a')$ is a constant with the value β_{km}. Therefore $\beta_i(a,a')$ can be represented by a matrix of constants $[\beta_{ikm}]$ which is called the 'who acquires infection from whom' or WAIFW matrix. The components of this matrix are too complex to measure directly, but by using the above definition of the force of infection it is possible to estimate values of β_{ikm} by an indirect method (Anderson and May (1984)). The estimated forces of infection are used to generate equilibrium age distributions for each of the five age classes in the model. From the age distribution for the infectious (Y) class, the total number of infectious individuals in each age class (Y_k) is calculated. The total population N is estimated from demographic tables. Because the WAIFW matrix only contains n different β_{km}'s the set of linear equations (6.1)-(6.5) can be solved for the n different β_i's. Appendix C shows the WAIFW matrices used in this study.

(b) The Force of Infection (FOI).

The force of infection $\lambda_{i(a,t)}$ plays a major part in disease dynamics since it governs exactly the rate at which new cases are generated. The force of infection is defined as 'the rate at which a susceptible individual acquires infection' for each virus strain.

It is this force of infection which characterises the model as outlined in section 2.1, and the importance of the relationship between the force of infection and the size of the susceptible population cannot be understated. This relationship has been defined in the following way for this study, assuming $\lambda_i(a,t)$ is constant over the age ranges determined in the definition of $\beta_i(a,a')$;

$$\lambda_{ik}(t) = \Sigma^m_{k=1} \beta_{ik}(Y_{ik}(t)+V_{ik}(t)) \tag{6.6}$$

$$\text{Where } Y_{ik}(t) = \int_0^\infty Y_{ik}(a',t) \, da'; \text{ and } V_{ik}(t) = \int_0^\infty V_{ik}(a',t) \, da'$$

In this classical age-structured deterministic model (see also Kermack and McKendrick (1927) and Bailey (1975)) X, Y, V and N represent the densities of the susceptible, the two infectious and the total populations respectively (as defined earlier). In this case the sub-populations which are immune to one strain only are considered to be susceptible to all intents and purposes when dealing with the second strain, if cross-immunity occurs a proportion (dependant on the value of the cross-immunity coefficient) of the individuals infected become immune to both strains, and move directly to the W sub-population. It is assumed that individuals convert from the susceptible to the infected classes at a rate proportional to the product of the density of susceptible and infectious individuals (i.e. assuming strong homogeneous mixing). Age-dependant principles deal in numbers of individuals and therefore it is necessary to use numerically defined sub-populations in any simulations, as opposed to proportions of the whole population as used in chapter 2.

The forces of infection can be estimated from serological data by the catalytic infection model described in chapter 5. Once age dependant values for λ_i have been determined it is possible to construct an age-structured population at equilibrium for which the β_{ikm} values can be determined by solving the matrix of simultaneous equations outlined above in the de-

scription of the force of infection (Anderson and May (1984)). All values for the age-dependent forces of infection are measured per year.

(c) The Cross-immunity Coefficient.

The cross-immunity coefficient used in this age-structured model is identical to that described in chapter 2 for the non age-structured model. Thus it represents the ratio of the number of individuals infected with one strain of the virus to the number of individuals infected by the other. Values for this parameter are estimated in the same method as that described in chapter 2.

(d) The Recovery Rate.

The recovery rate is determined from the reciprocal of the infectious period (in days) and in this study is given the value of $0.166 \, \text{day}^{-1}$ (assuming an average infectious period of 6 days).

(e) The Mortality Rate.

It is assumed that mortality is constant for the analysis of the non age-dependant model, and is thus the reciprocal of the average life expectancy, taken to be 75 years, giving a value of $0.013 \, \text{year}^{-1}$ for μ. In this chapter, where the population is age-structured, an age-dependant step function is used to represent the mortality rate. The step function leads to a 'type II' survival curve, such that all individuals live to the age of 75, and then all individuals die. This type of function is considered to be realistic for the population of a developed country (Anderson and May (1983a)).

6.2. Equilibrium Properties of the Age-structured Model.

The full partial differential equation model is too complex to be treated analytically, and because of this its solution is investigated numerically (see section 6.4.). However computer simulations of this type give limited insights into the models behaviour. The analytical treatment of a simplified version gives very useful information, which can often be overlooked by those unfamiliar with a model's characteristics. The results of such an analysis are com-

plimentary to those obtained from numerical treatment, and can present various properties of the model's behaviour in easily understood formulae.

In order to simplify equations (6.1)-(6.5) it is assumed that over a long period of time the force of infection tends to a constant, and the numerical structure of the population is at equilibrium. Thus the model becomes a set of ordinary differential equations, describing the constant age distribution. Under this assumption, that $\lambda_{i(a,t)}$ and $\mu_{(a)}$ are constant over given age ranges, the equilibrium values of the partial differential equations are found by setting all time derivations to zero. This then gives us the following expressions for the equilibrium age distributions of individuals in each of the various classes in terms of the parameters;

$$dx/da = -(\mu_{(a)}+\lambda_1+\lambda_2)X_{(a)} \tag{6.7}$$

$$dy_i/da = \lambda_i X_{(a)}-(\mu_{(a)}+\gamma_i)Y_{i(a)} \tag{6.8}$$

$$dz_i/da = (1-\sigma)\gamma_i Y_i-(\mu_{(a)}+\lambda_j)Z_{i(a)} \tag{6.9}$$

$$dv_i/da = \lambda_i Z_{j(a)}-(\mu_{(a)}+\gamma_i)V_{i(a)} \tag{6.10}$$

$$dW/da = \sigma\gamma_1 Y_{1(a)}+\sigma\gamma_2 Y_{2(a)}+\gamma_1 V_{1(a)}+\gamma_2 V_{2(a)}-\mu_{(a)}W_{(a)} \tag{6.11}$$

$$dN/da = -\mu_{(a)}N_{(a)}$$

In this case the force of infection is taken to be:

$$\lambda_i = \beta_i \int_0^{\omega} [Y_{i(a)}+V_{i(a)}]da \tag{6.12}$$

Following Anderson and May (1983) we can solve these equations (see also Dietz (1975) or Bailey (1975)) thus:

$$X_{(a)} = N_{(0)}exp(-(\lambda_1+\lambda_2)a- \int_0^a \mu_{(s)}ds) \tag{6.13}$$

$$N_{(a)} = N_{(0)}exp(- \int_0^a \mu_{(s)}ds)$$

For simplicity only the results for the susceptible and total populations are shown.

To successfully consider the theory behind the derivation of expressions for the reproductive rate of the virus, R_0, and the average age at first infection (a), with two types of sur-

vival function (types I and II as considered in chapters 2 and 6 respectively), it is necessary to introduce a set of 'starred' variables and the function $\Phi(a)$, such that

$$\Phi(a) = \exp(-\int_0^a \mu(s)ds)$$

(6.14)

and therefore

$$X_{(a)} = X^*_{(a)}\Phi_{(a)}$$

and $N_{(a)} = N^*_{(a)}\Phi_{(a)}$

This is done so that terms involving $\mu_{(a)}$ are no longer present (since they are incorporated in the general survival term $\Phi_{(a)}$), enabling simple equations to represent both $X_{(a)}$ and $N_{(a)}$ in the following analytical solutions, and still allow relatively complex expressions to represent the survival functions under consideration, giving the following expressions for the population components;

$$X_{(a)} = N_{(0)} \exp[-(\lambda_1+\lambda_2)a]\Phi_{(a)}$$

(6.15)

$$N_{(a)} = N_{(0)}\Phi_{(a)}$$

If, as before, we consider the population of susceptible individuals as a fraction of the total population we have the following;

$$x_{(a)} = X_{(a)}/N_{(a)}$$

which, by simple division, gives us

$$x_{(a)} = \exp(-\int_0^a \lambda_1(s)+\lambda_2(s)) \, ds.$$

(6.16)

Therefore, the fraction of the population susceptible to the influenza virus is determined by the sum of the individual forces of infection for each strain of virus. Using these general terms for X and N outlined above it is possible to consider expressions for the basic reproductive rate, R_0 (as defined in section 6.3.), and the average age at first infection, A. These are dealt with in the next section.

6.3. Derivation of useful expressions.

In this section expressions are for the hypothetical situation where two strains of the same virus co-exist in an endemic equilibrium. Thus the expressions derived have theoretical meaning only, although it is possible that this situation could occur. However, due to the epidemic nature of the influenza virus it is far too complex to derive expressions for the more realistic situation where the strains only co-exist for a very short time period, during which time the various components of the population are continuously changing. However, these expressions do give an indication of the relationship between various parameters involved in the transmission of two co-existing strains.

The two expressions that are derived in this section are those for the basic reproductive rate, R_0 , and the average age at first infection, A of a virus when two strains co-exist at an endemic equilibrium.

6.3.1 The Basic Reproductive Rate.

The concept of the basic reproductive rate (R_0) was introduced in chapter 2, where various defintions were proposed for the three scenarios which are observed for co-existing virus strains. In this chapter, due to the complexities of the model under consideration, only the situation where two strains co-exist to give an overall basic reproductive rate (R_0') for the virus is considered. The argument follows that of Anderson and May (1983) for a single strain at an endemic equilibrium, but is enlarged to consider two strains whic co-exist.

It is possible to obtain the total number of individuals in any one class, by aggregating over all ages:

$$X = \int_0^\infty X_{(a)}\, da$$

$$N = \int_0^\infty N_{(a)}\, da$$

If we consider equations X and N (as expressed above in terms of $\Phi(a)$), by simple integration expressions can be derived for the total number of individuals in the two classes at equilibrium, which are;

$$X = N_{(0)} \int_0^\infty \exp[-(\lambda_1+\lambda_2)a]\Phi_{(a)}\, da \qquad (6.17)$$

and,

$$N = N_{(0)} \int_0^{\infty} \Phi_{(a)} \, da$$

Having determined the expressions for the total numbers of individuals in both the infected population and for the total population it is possible to consider the two different types of survival function; type I and type II.

(a) Type I survival.

For type I survival it is assumed that the mortality rate μ is a constant for all ages, which can be represented;

$$\mu = 1/L$$

where L is the average life expectancy of the population (assumed to be 75 in this study) as described for chapter 2.

Type I survival produces the following expression for Φ;

$$\Phi = \exp(-\mu a)$$

which, following Anderson and May (1983), and integrating, leads to the expressions

$$X = \frac{N_{(0)}}{\lambda_1 + \lambda_2 + \mu}$$

$$N = \frac{N_{(0)}}{\mu}$$

Considering the population in terms of fractions, such that

$$\bar{x} = X/N,$$

an expression can be derived for the net fraction susceptible in the population at equilibrium thus;

$$\bar{x} = \frac{\mu}{(\mu+\lambda_1+\lambda_2)}$$

This gives an expression for the susceptible proportion of the population which differs only slightly from that described by Anderson and May (1983) for a single strain, and determines that with a constant mortality rate for all ages, $\bar{x}$ is inversely proportional to the sum of the separate forces of infection for each strain, and hence

$$R_0' = 1 + [(\lambda_1+\lambda_2)/\mu] \tag{6.18}$$

(b) Type II survival.

For type II survival, which is a more realistic 'step' function (that is all individuals live until age L and then die), the following expressions hold true;

$$X = \frac{N_{(0)}.\ 1-\exp[-(\lambda_1+\lambda_2)L]}{\lambda_1+\lambda_2}$$

$$N = N_{(0)}L$$

and hence

$$x = \frac{1-\exp[-(\lambda_1+\lambda_2)L]}{\lambda_1+\lambda_2}$$

which gives

$$R_0' = \frac{(\lambda_1+\lambda_2)L}{1-\exp[-(\lambda_1+\lambda_2)L]}$$

and

$$R_0' = \frac{(L/A)}{[1-\exp(-L/A)]}$$ (6.19)

This gives us an identical result as that obtained by Anderson and May (1983) using only a single strain and hence a single force of infection. It shows that the basic reproductive rate can be determined from simply the life expectancy (L) and the average age of infection (A) which is derived for two strains in the next section.

6.3.2. Average Age at Infection (A).

As shown earlier, R_0 is dependent (by definition) on L and A, not directly on variations in λ, μ, γ, or σ, therefore it is more relevant to determine the changes in A, unless looking at derivations from the ordinary differential equation system.

By following similar studies on single strain dynamics (Anderson and May (1991)), it is possible derive an expression for A from equation (6.18), if it is assumed that the force of infection is constant, such that

$$A = \frac{\int_0^\infty a\,[\lambda_1(a)+\lambda_2(a)]x(a)\,da}{\int_0^A [\lambda_1(a)+\lambda_2(a)]x(a)\,da}$$

which gives, by substitution with partial integration to the numerator;

$$A = \frac{\left[-a\,\exp[-\int_0^a(\lambda_1(s)+\lambda_2(s))ds]\right]_0^\infty + \int_0^\infty x(a)\,da}{\left[-\exp[-\int_0^a(\lambda_1(s)+\lambda_2(s))ds]\right]_0^\infty}$$

$$A = \int_0^\infty x(a)\,da$$ (6.20)

If a term is now introduced which represents the 'probability density function' for the age distribution of infected individuals, $g(a)$, where

$$g(a) = x(a)/A$$

and $$\int_0^\infty g(a)\,da = 1$$

Then, the average force of infection can be expressed;

$$\lambda_1 + \lambda_2 = \int_0^\infty (\lambda_{1(a)} + \lambda_{2(a)}) g_{(a)} \, da$$

$$= \frac{\int_0^\infty (\lambda_{1(a)} + \lambda_{2(a)}) x_{(a)} \, da}{A}$$

$$= 1/A$$

Therefore the average forces of infection, combined, are equal to the reciprocal of the mean age of infection:

$$A = \frac{1}{\lambda_1 + \lambda_2} \qquad (6.21)$$

6.4. Numerical analysis.

Using a step length of one week (for a simulation of twenty years) or one day (for a run length of one year), Euler's method is used to solve the equations (6.1)-(6.5) along the characteristic lines t = a + constant. The initial conditions are set by allowing the system to reach an equilibrium and then calculating the age-dependant forces of infection that are defined by the base-line parameter set. After the equilibrium solutions have been found the elements of the WAIFW matrix are calculated by the method described in section 6.5.2(a). After the WAIFW matrix has been calculated, in all subsequent steps it is used to determine the forces of infection. This can be done using equation (6.6) with the vector describing the number of cases by age at the last time step.

The estimates of the rates of infection derived from serological data (chapter 5) show a basic convex shape as age increases. The force of infection is low in the young children (0-4 years of age) and early adult (15+ years of age) classes (Table 5.2). However, each method of estimation of the forces of infection, either case notification or serological, gives considerably varying patterns within the basic convex shape, with varying magnitudes. If an average 'base-line' force of infection is estimated for each age group from all sources it is possible to use these values in the numerical analysis of the age-structured model described

earlier. These 'average' values for the age dependant forces of infection are shown in Table 6.1(b).

When these values, and the other parameters as shown in Table 6.1, are used in the age-structured model the epidemic generated can be considered in terms of age and time. The course of the two strains through the population can be seen in Figs. 6.1 and 6.2. This shows the same pattern of epidemics as that generated by the age-independent model described in chapter 2. One virus strain is predominant, causing more cases in a shorter time span than the other strain, which lasts longer but produces less cases. From Fig 6.1 it can be seen that the age-structure of the epidemic is much as would be expected, with younger age groups (less than 15 years old) being affected more by both strains than the older age groups (greater than 15 years old).

Table 6.1(a);The base-line parameters used in the numerical analysis of the age-structured model. The recovery rate is held constant for both strains, as in chapter 2.

Parameter	Symbol	Value
Time Step	t	1 week
Death rate	μ	1.3x10-2 year-1
Recovery Rate	γ_i	0.166 day-1
Age-dependant FOI	λ_i	See table 6.1(b)
Cross-immunity Coefficient	σ	0.25

Table 6.1(b): Age dependent forces of infection used in the numerical analysis of the model. All values are based on the average values of those age dependent values shown in Table 5.2

Age group (Years)	Force of infection estimates (λ_i) (year-1)	
	(Strain 1)	(Strain 2)
1-5	0.055	0.110
6-10	0.045	0.100
11-15	0.037	0.075
16-55	0.027	0.035
56-75	0.000	0.000

0.3

Proportion of

Population

Infected.

Age (Years).

0.3

Age (Years).

Time (Weeks).
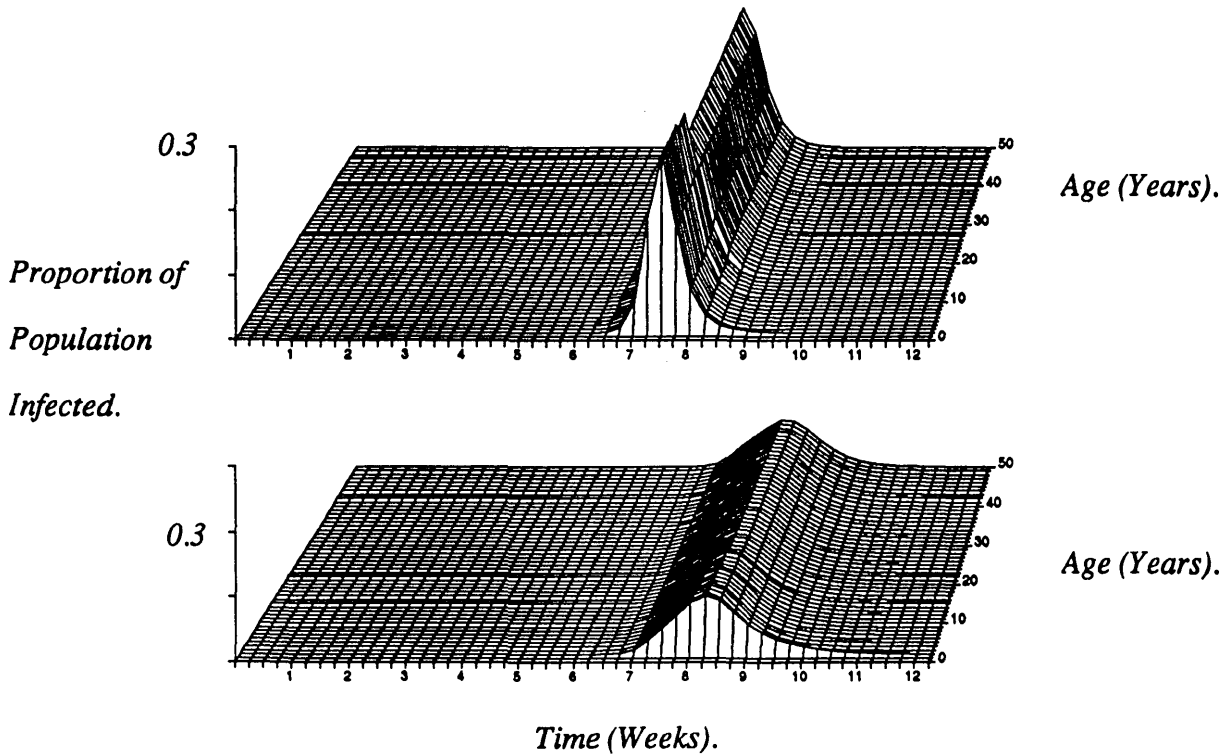
Figure 6.1; The proportion of the population infected by the two strains of the virus show-
ing the time scale of the epidemic. The top graph shows the cases generated by infection
with virus strain Y, and the lower graph shows the cases generated by infection with
strain V. The parameter values are detailed in Table 6.1.

0.3

Proportion of

Population

Infected.

Time (Weeks).

0.3

Time (Weeks).

Age (Years).
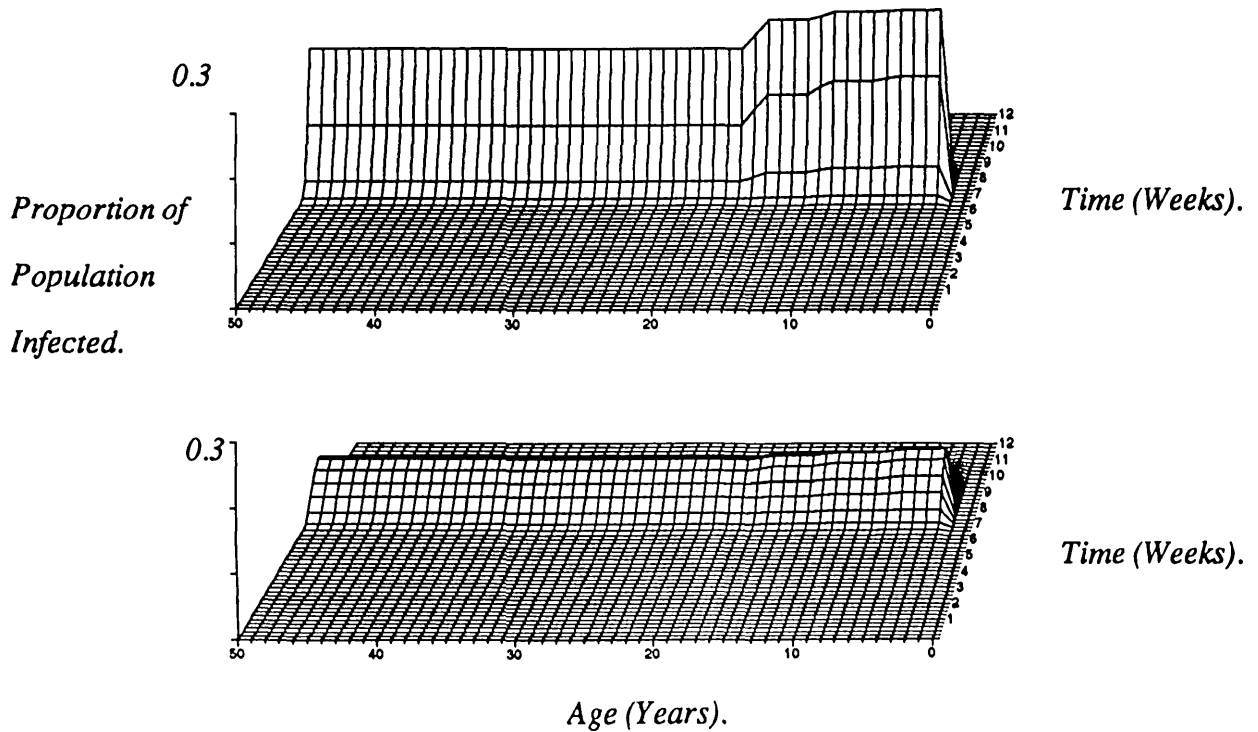
Figure 6.2; The proportion of the population infected by the two strains of the virus show-
ing the age structure of the population. The top graph shows the proportion of the popu-
lation infected by strain Y, and the lower graph the proportion infected with strain V.
The parameter values are detailed in Table 6.1.

It is also possible to construct a 'serological' surface showing the proportion of the population which has been exposed to the virus (Fig 6.3). Similar surfaces can be constructed to show immunity to each strain of the virus, which are identical in shape to the overall serological surface, but of a lesser magnitude. The most striking aspect of this surface is the extreme proportion of the population which becomes immediately immune to the virus. Approximately 75% of all individuals under the age of 15 are immune to infection after the initial epidemics.

This situation changes through time in a predictable manner. The immune individuals move through the age classes as time passes, and are replaced by susceptible individuals in the less than 1 year age group (ie new-born individuals). This is clearly shown in Fig 6.4 which shows the proportion of susceptible individuals increasing through time. This proportion reaches the threshold level, which would allow a virus to cause a new epidemic, only after such a long period of time that the virus strains are no longer maintained in the community. Therefore the model predicts that after one 'boom and bust' season, or epidemic,
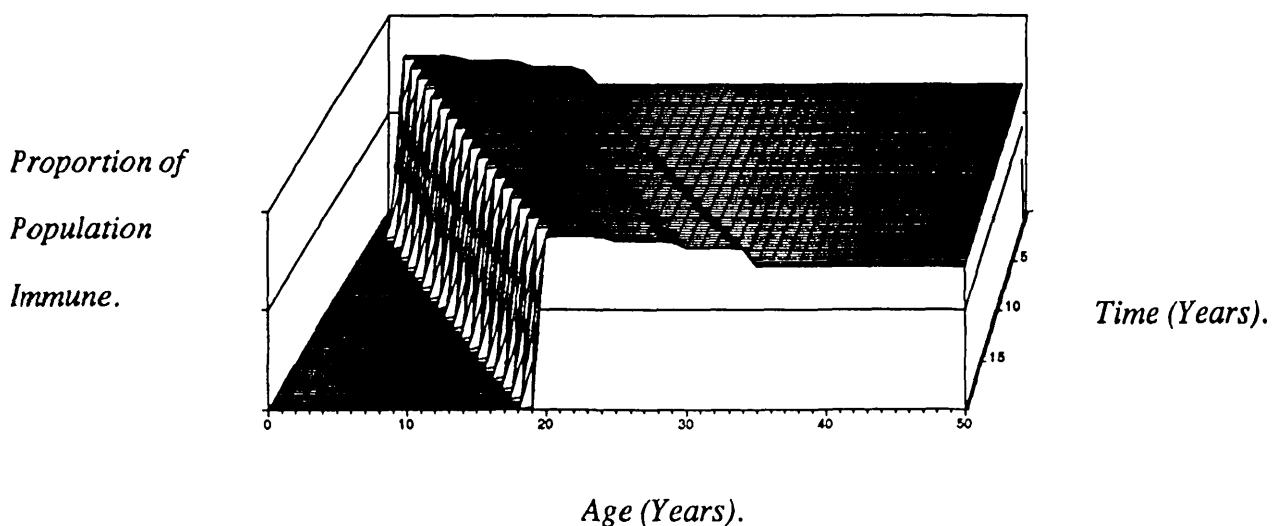
Figure 6.3; The proportion of the population immune to both strains of the virus, showing the age structure and progression through time. The parameter values are detailed in Table 6.1.

the virus strain is unable to cause a second epidemic. The overall decline in the proportion of the total population immune to the strains of the virus can be seen in Fig 6.5 which shows in the change in the immune proportions through time.

From further numerical analysis it appears that only forces of infection which are several orders of magnitude lower than any of those observed in serological or case notification studies are able to generate a situation where the virus strains reach an endemic equilibrium in the population. This is also possible when the initial populations are set at levels which are never observed in the epidemiological survey (see chapter 5), namely when the proportion of immune individuals in the population is twice as large as that of susceptible individuals.

Proportion of

Population

Susceptible.   *Time (Years).*

Age (Years).
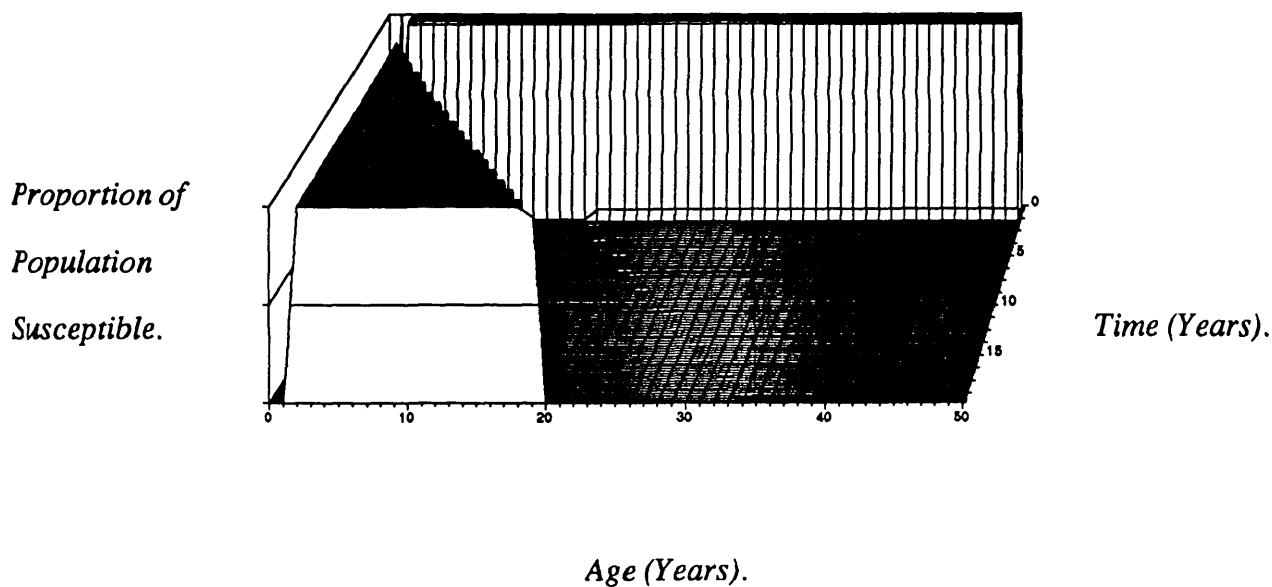
Figure 6.4; The proportion of the population susceptible to both strains of the virus, showing the age structure and progression through time. The parameter values are detailed in Table 6.1.

Proportion of

Population

Immune.

Time (Years).
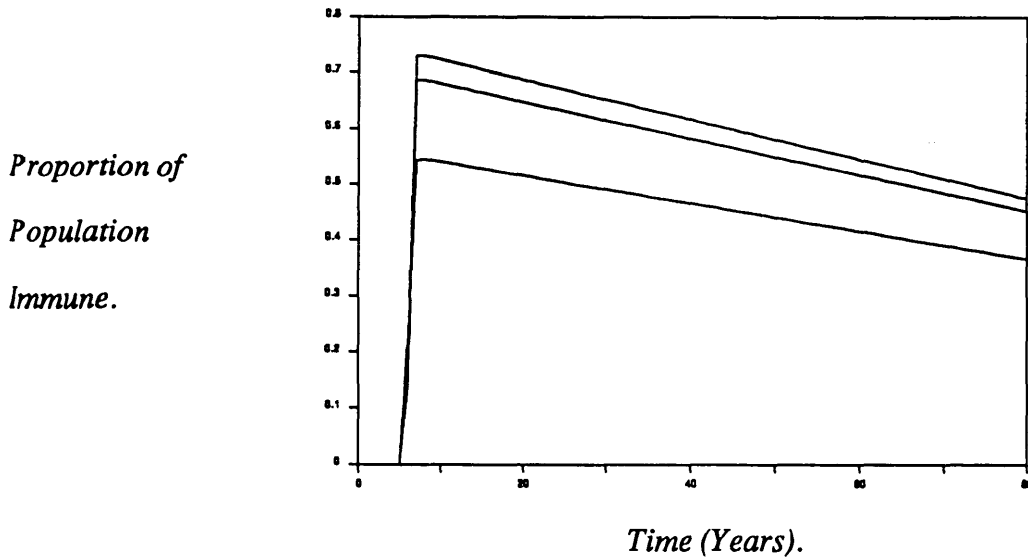
Figure 6.5; The proportion of the population immune to the strains of the virus. The top line shows the proportion of the population immune to both strains, the middle line shows the proportion immune to strain Y, the lower line shows that proportion immune to V. The parameter values are detailed in Table 6.1.

Proportion of

Population

Immune.

□   Type A1   +   Type C1

Age (Years).
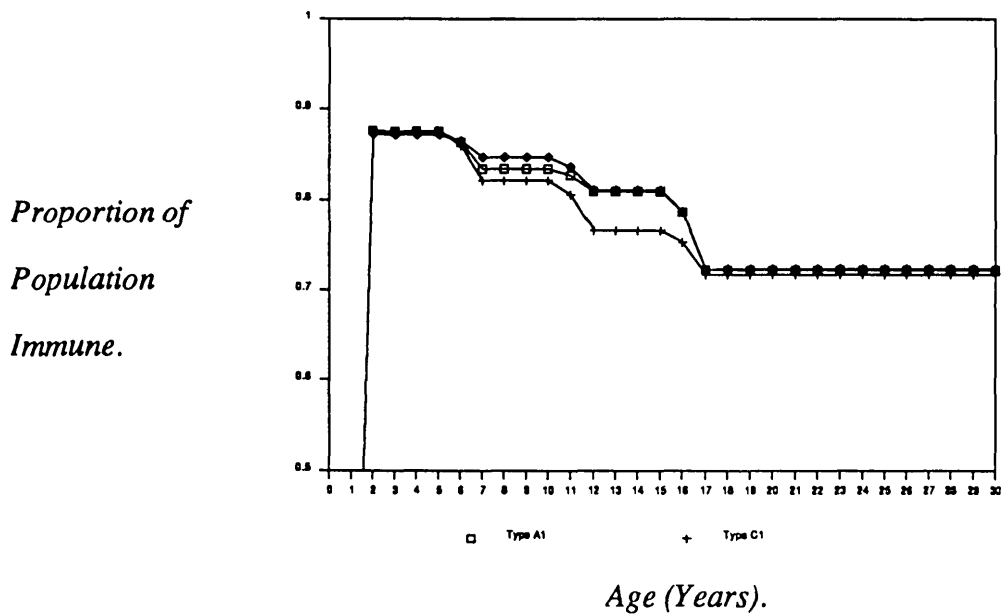
Figure 6.6; The effect on different types of mixing between infectious and susceptible individuals on the age structure of the immune proportion of the population. The top line shows the matrix with configuration A, the middle line configuration B and the lower line configuration C (see text for details). The parameter values are detailed in Table 6.1.

Table 6.2; Observed and Predicted values for the Basic Reproductive Rate and Average age at first Infection for two co-existing strains, assuming all parameters are as detailed in Table 5.3.

Parameter	Observed	Predicted
Reproductive Rate*	2 - 4[+]	2.4
Average Age (Years)	20-30[+]	6[++]

*Estimated from equation (6.19).

[+]Data from table 3.1

[++]Estimated from equation (6.21).

In the preceding analysis the 'who acquires infection from whom' (WAIFW) matrix is that which is considered to be the major route of transmission for many directly transmitted viral and bacterial infections, namely within schools (Glezen (1984) and Anderson and May (1985)). The configuration of the matrix describes unique transmission coefficients (β) to describe intense contact within child age groups, and less intense contact between and within other age groups. Given the high observed forces of infection in the 5-10 year-old age groups and the 10-15 year-old age groups it seems likely that susceptibles within these groups usually acquire infection from infected individuals of their own classes. These notions are captured by configuration A of the matrix. However, it is useful to test the sensitivity of the model predictions to variation in the contact configuration. Two other matrices are thus also considered (see appendix C for all configurations). The three matrices considered A, B and C are the only biologically feasible configurations to represent contact. It can be seen that the mixing within and between age groups has little effect on the immune status of the population, possibly due to the rapid transmission of the virus between the human hosts, which causes a large number of individuals to become infected over a short time period and hence

a swift conversion of those individuals to the immune class (Fig 6.6) essentially regardless of age.

6.5. Discussion.

The equilibrium analysis of this model, which incorporates an age-structured population, gives rise to some interesting expressions for the basic reproductive rate of the virus (R0), and the average age of infection (A) for the situation where two strains of the virus co-exist. However, both derived expressions have two major deficiencies. First, all steady-state analysis of the model assumes that the virus strains co-exist in an endemic equilibrium, which has been shown in chapters 3 and 5 to be incorrect for the influenza virus. Second, the final expressions for both these epidemiological terms involve parameters which cannot be estimated unless the transmission of the individual virus strains throughout a host population can be observed in isolation from any other strains of the influenza virus. This constraint is impractical and unfeasible at the present time, therefore, although these expressions are interesting in purely theoretical terms, they cannot be used in this study to give estimates of either the basic reproductive rate (R_0) or the average age of infection (A) for the influenza virus when two strains are in co-circulation. They do, however, highlight the need for further analysis of this sort to be performed both in terms of an epidemic situation, and considering the case where two strains co-exist.

The most interesting aspect to arise from this model is that the number of infected individuals is not sustained for more than one season. Thus, although the susceptible population is allowed to increase (due to the recruitment of new-born individuals) the density of infected individuals is not sufficient to cause a new epidemic within a time period of at least 50 years. Therefore, after a time period spanning approximately one generation the population will once more be entirely susceptible to the viruses which caused the initial epidemic. Before this time period elapses, however, herd immunity to that particular strain is virtually complete. This is in support of the findings of many workers (Chakraverty (1986), and Glezen, Couch and Six (1982) for example) that there are no cases in the off season. The numerical analysis of the model also supports the results of chapter 5 that immunity to a par-

ticular strain of the influenza virus is sustained for at lest one generation of the host population.

Unfortunately, the numerical analysis of this model does not allow a detailed enough stratification between age groups (as a result of the manner in which the parameters were estimated in the previous chapter) at an early stage in the introduction of the virus strain. It would be useful to develop the method of parameter estimation, and numerical analysis, to examine the short term (i.e. in terms of days) dynamic interactions between the age groups infected with the virus strains.

The numerical analysis of this model shows the importance of the cross-immunity coefficient coupled with he age-dependent forces of infection in the production of the typical 'bi-modal' epidemic curve. It also goes some way to explaining the disparity in results which appear between various epidemics, with regards to the size of the epidemic, in terms of a previous immunity to the sub-type circulating. One of the problems which is highlighted by this model is that associated with the epidemic nature of the influenza virus. It is not within the scope of this thesis to derive expressions which represent the introduction of a virus into a totally naive population, and as a consequence the estimation of values such as the average age of infection rely upon the theories which have been derived for use with viruses which are at an endemic equilibrium. Results thus tend to show some disparity with the observed values obtained in chapters 3 and 5. More work of this sort is therefore needed.

Chapter Seven: Summary Discussion.

In this chapter the theories currently considered to apply to the transmission dynamics of the influenza A virus are discussed, and some of the problems that are associated with the study of the virus are highlighted. The research described in chapters 1 to 6 is summarised and the ideas that have arisen from the various components of this study are discussed in relation to the prevailing theories. In doing this, the deficiencies inherent in some aspects of the data and its analysis that have become apparent during the course of this work are highlighted. A variety of new problems are revealed when the results of each chapter are brought together, and these provide several different perspectives on the study. Finally, the major conclusions of the study are presented and future research needs discussed.

There are currently two main hypotheses to account for the persistence of the influenza virus in humans. One theory is that the viruses are maintained entirely in human populations by serial transmission from person to person, possibly requiring the annual transfer of the virus population between Northern and Southern hemispheres (Longini et al. (1984)), latency in some individuals (Hope-Simpson and Golubev (1989)) or periodic antigenic change (Webster et al (1982)). The second hypothesis is that the influenza virus cannot be maintained in human populations alone and that the survival of the virus is dependant upon circulation in other mammalian or avian hosts (Andrewes (1942)). To fully understand the problems of influenza A persistence, it is crucial to appreciate the seasonality of influenza A activity, as outlined in chapter 3, since it is undeniably some seasonal factor which triggers the onset of an epidemic with a 'new' strain. The plausibility of continuous transmission through a heterogenous population is supported by the variation in influenza activity between countries in the same hemisphere (Thacker (1986)).

The role of birds and mammals in the influenza cycle has long interested researchers. Evidence for interaction between influenza viruses circulating in human and animal populations is strong, although somewhat circumstantial. Laboratory evidence includes isolation of viral strains with shared peptide sequences from humans and from swine, equine and avian species (Webster et al. (1982) and Kilbourne (1960)). It has also been suggested that the migratory habits of birds could explain the emergence of the same virus in different geographic

locales (Andrewes (1942)). However, the evidence for an essential interaction between humans and other animals for persistence of the influenza virus remains circumstantial.

At various stages in this thesis comparisons have been made between the influenza virus and the measles virus, generally with respect to the similarities between the transmission of the two strains. The models presented in this thesis assume that transmission of the influenza virus is by direct contact between infected and susceptible individuals. It is considered that this contact, combined with the antigenically diverse nature of the influenza virus, leads to its persistence within human populations. However, the epidemiological study of influenza is somewhat more difficult than that of measles. The viruses of measles and influenza belong to different branches of the myxoviridae (para- and ortho- respectively). More importantly, there are differences in at least two key aspects of the infectious agent-host interaction which make the epidemiological study of influenza inherently more complicated than that of measles. First, while influenza infection does cause 'typical' disease, similar disease can also be generated by other agents, and many infections are asymptomatic or cause non-typically mild disease. Thus, the identification and specific recognition of infection require laboratory assistance and is difficult to achieve except in a small defined population under continuous virological surveillance. Second, post influenza infection immunity, because of the continual antigenic change of the virus within human populations, can be considered as solid for no longer than a single epidemic season, since immunity to a strain in one season does not necessarily confer immunity on a new strain in the subsequent season. While a general decline over time in resistance to reinfection is typical of other respiratory myxoviruses (parainfluenza and respiratory syncytial viruses) such a decline is effectively, but unpredictably, enhanced by the changing antigenic character of type A influenza virus.

The aim of this study was to determine what role, if any, cross-immunity has in determining the transmission dynamics of co-existing viruses in human populations. In order to explore this problem several strains of influenza were studied, both in terms of the notifications of cases of illness, and with respect to the evidence of exposure to the virus as revealed by serological studies. As a consequence of similar research by many other workers (Hope-Simpson (1979), Glezen (1982), Anderson and May (1985) and Nokes (1990) for example) another objective of this study was to determine whether any age-dependant effects were re-

sponsible for some of the longitudinal patterns of infection and incidence observed. It was necessary, therefore, to consider both case notifications and serological data in an age-stratified manner for both subtypes (and hence various strains) under consideration. This lead to the discovery that the force of infection is age-dependant and to the hypothesis that the appearance of the typically bi-modal shape of the epidemics may well be due to the influence of cross-immunity between the two strains.

The serological assays were performed on several large sera sets for two main reasons. First, to help investigate the epidemiology of co-existing strains, and to determine whether cross-immunity is an important determinant of observed epidemiological patterns. Second, it enabled parameters to be estimated for use in mathematical models of the transmission dynamics of the influenza virus. The concepts of cross-immunity and age-dependent rates of transmission could thus be explored by way of two mathematical models; a basic model which was used to explore the dynamics of the co-existing strains through time, and a more complex model, which incorporated age structuring in the population, and hence allowed age-dependency of transmission to be considered.

The numerical analysis of the simple non-age-structured model has given some useful insights into the dynamics of co-existing strains of the same virus. In terms of the relationship between two strains of different infectivity which co-exist, assuming no cross immunity occurs, the simple model generates patterns similar to those observed from case notification data. However, from Fig. 2.9 it can be seen that the cross-immunity coefficient may well have some considerable bearing on the transmission of co-existing strains. In particular the simple model suggests that cross-immunity causes an alteration in the behaviour of co-existing strains in terms of the transmission efficiencies of each strain when considered together or in isolation (Fig. 2.5). Thus, by introducing an element of cross reactivity between strains, competition for susceptible individuals is generated (which causes irregular patterns with respect to the reappearance of each strain of the virus (Figs. 2.6 and 2.7)). In terms of the time scale which can be imposed on the reappearance of a particular strain of a virus, it has been shown (numerically) that the same dynamics are observed between co-existing strains even if the appearance of the second strain is delayed by as much as 25 years. Not until the population of susceptible individuals has been fully replenished by new births will the ef-

fects of cross-immunity be lost. The relationship between the force of infection and the size of the epidemic shows clearly that the cross-immunity coefficient is not necessary to obtain patterns that are observed from case notification data. However, cross immunity limits the size and duration of any given epidemic (Fig. 2.9). If there is a significant role played by strain specific cross immunity it may well be as a limiting factor in both the size and shape of the epidemics, and also in the number of strains which can co-exist in any one season.

One of the problems highlighted by both models is that associated with the epidemic nature of the influenza virus, and as a consequence the estimation of values such as the average age of infection rely upon the theories which have been derived for use with viruses which are at an endemic equilibrium. Results thus tend to show some disparity with the observed values obtained in chapters 3 and 5. More work is therefore required on the correct procedure to be employed in the estimation of parameters such as R_0 and the average age at infection (A) for epidemic infections. The numerical analysis of the model without age structure, which assumes cross-immunity, gives an excellent correlation to the data collected from case notifications, as shown in Fig. 7.1. The case notification data shown consists of two strains of the same sub-type co-existing during the same epidemic season. Although this does occur, it is not very commonplace, and is shown here merely to illustrate that the model gives epidemics of the appropriate magnitude and time scale. As shown in chapter 2, the same effect is observed over a number of epidemic seasons, thus the cross-immunity conferred on one strain by a similar strain may be long lasting, and may cause the same effect many years later. Both the forces of infection derived for each strain and the cross-immunity coefficient assumed for the two strains generate predictions that crudely mirror the observed epidemics. It is not possible at this stage to state whether or not cross immunity plays an essential role in the generation of the bi-modal infection curves observed, or whether these patterns are simply a result of varying forces of infection (and hence the values of the strain-specific basic reproductive rate R_{0i}) between the strains of the virus. However, it can be stated that if cross immunity does occur between strains of the same virus then this effect will considerably alter the dynamics of the co-existing strains.

When the model is considered with age-structure it produces 'age serological profiles' which closely resemble those derived from serological analysis (compare Figs 5.4 and 7.2).

0.3   80

Proportion of

Population infected

(From model)

Number of

Individuals infected.

(Case notifications).

0   20   40   60   80   100

Time (Days).
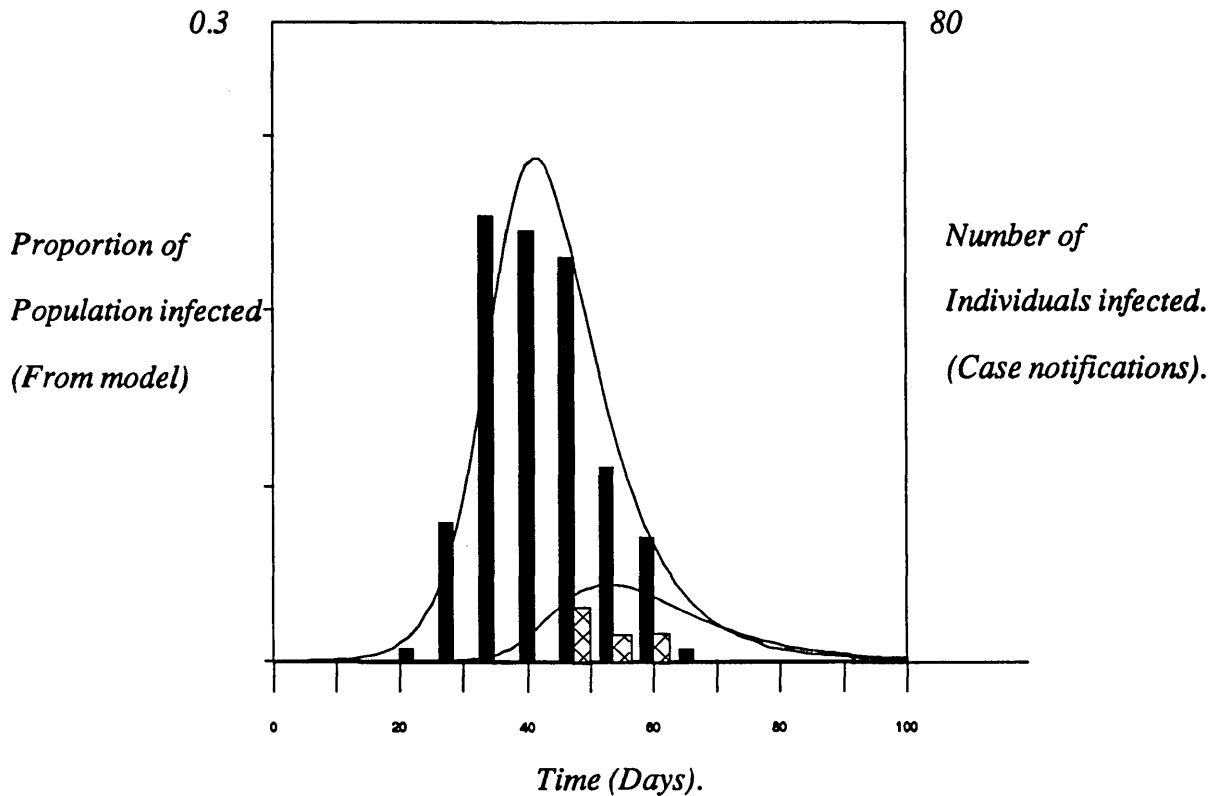
Figure 7.1. The results from the numerical analysis of the non-age-structured model (with b = 0.4, s = 0.25 and other parameters as in Table 2.1), represented by the solid line, superimposed on the case notification data for two strains of the H1N1 subtype (data from Glezen (1982)), shown by the filled bars. The solid bars are numbers infected with A/USSR/90/77 and the cross-hatched bars those infected with A/Brazil/11/78.

The age structured model gives a good approximation to the overall decay in seropositivity observed over time. The model is not able to sustain a significantly large population of infected individuals to cause another epidemic within the time span of one generation of the host population, due to the virus strains being introduced into a population consisting entirely of susceptible individuals. The rate of infection is so rapid that almost 80% of the population becomes immune to the virus strains over the time period of the epidemic, thus preventing further epidemics of the same strains of virus. In this scenario a new epidemic could only be caused by an antigenically different virus strain. This leads to the hypothesis that the irregular temporal appearance of the epidemics, in terms of strain recycling, may
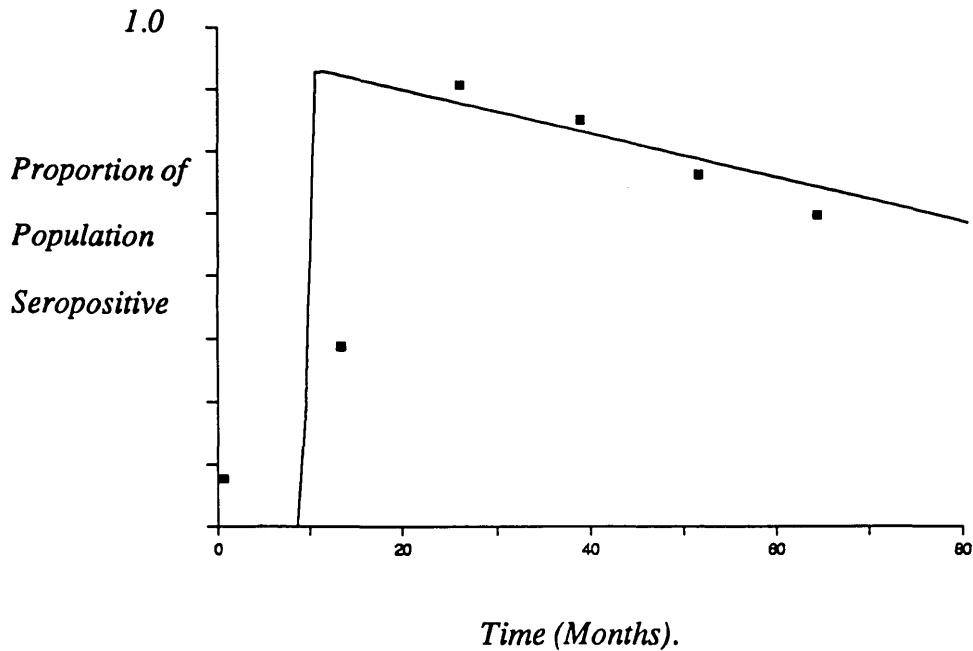
Figure 7.2. The results from the numerical analysis of the age-structured model (with parameters as in Table 6.1) are represented by the solid line. All age classes have been summed to give the resulting overall change in the proportion of the population positive through time. The filled squares are the results from the serological analysis shown in Fig. 5.5 for A/Hong Kong/68 (H3N2).

well be due to the influence of cross-immunity between the two strains. It appears from the numerical analysis of the mathematical model that there are two main effects of cross-immunity; less cases are involved in an epidemic caused by a later strain, which encounters a degree of immunity derived from an earlier infection of a similar strain; and as a result of the cross-immunity different forces of infection between the strains lead to markedly differing patterns of infection. Differing forces of infection of two co-existing strains, in the absence of cross-immunity, certainly can give similar patterns to the observed epidemic curves. However, the results from analysis of the model indicate that a more diverse set of epidemic patterns would be observed if this were the only causative force behind the generation of

new epidemics. Thus although cross-immunity between co-existing strains may not be the sole reason for the pattern of the epidemic curves, it is very likely that cross immunity between two similar strains, in combination with the force of infection, affects the appearance of influenza epidemics in large communities over both short and long time periods.

The bi-modal epidemic curves which were predicted from the numerical analysis of the mathematical model explored in chapter 2 are reflected in the case notification reports, which suggests that these epidemic curves are caused by the co-existence of two or more strains of the same virus. The most obvious aspect to arise from the study of the case notification data is that the influenza virus causes seasonal epidemics virtually every year, and that two sub-types of influenza invariably co-exist, with one of the sub-types appearing to be dominant (in terms of recorded incidence or seroprevalence) over the other (see Fig 3.2). It is probable that this is due to the first strain having a greater basic reproductive rate than the second, and thus generating more cases. The fact that a new epidemic can be generated each year by the same sub-types is due to mutation in the major antigen on the surface of the virus, the haemagglutinin protein, producing a new strain of sub-type each year (antigenic drift (Webster,R.G. et al. (1982)). The annual periodicity for the years studied (1978 to 1985) was due mostly to different strains of the H3N2 sub-type (Fig 3.11). Time-series analysis of case notification data was unable to uncover any longer term (other than seasonal) oscillations in incidence (Figs. 3.12 to 3.15). This can be explained by considering the equation (6.18) which states that the overall basic reproductive rate of a virus with two co-existing strains, R_0', is directly proportional to the sum of the forces of infection of the two strains. Therefore, with two strains circulating during the same season the reproductive potential of the influenza virus will always tend to be greater than that of an endemic myxovirus, such as measles, where the reproductive rate is proportional to a single force of infection. The resultant reproductive rate will also depend on the degree to which infection with one strain influences immunity to the co-circulating strain. The influenza virus, with co-existing strains, will thus tend to rapidly exhaust the pool of susceptible individuals in a population over a shorter time period than a single strain of virus. Therefore, in the case of influenza, where an antigenically 'new' viral strain appears in any given season, it can reasonably be assumed that the proportion of the population susceptible to the new strain is approximately 100%.

This would, therefore, have the effect of keeping the proportion of susceptible individuals (and hence immune individuals) at an approximately constant level for the beginning of every epidemic season. If this were the case, no regular cycles would be maintained for any population which was exposed to the ever-changing influenza virus. This would also be indicated by the lack of any periodicity in the average age at first infection. It is important to note that the results from the numerical analysis of the simple mathematical model, presented in chapter 2, suggested isolated irregular epidemic recurrences of differing strains.

The average ages of infection for the strains which were co-existing during the season 1982/83 (A/Eng/333/80 and A/Bel/1/81) are dissimilar to those observed for influenza A regardless of strain (somewhere between 20 and 30 years old); both values are much lower than those found for influenza A regardless of strain. This may be an artefact of the grouping of the data by age (i.e. the chosen age ranges). Most of the observed cases occur in the 15-44 year age group, which is in agreement with the average ages of infection of between 20 and 30 years old suggested in this study and in others (Table 3.1). Further stratification of this age group is required to determine which elements in this group are responsible for these age effects. Unfortunately the data does not allow this, analysis which would be useful to determine whether the observed high numbers of infected individuals in this age group are in fact of approximately school age. This would support the findings of Glezen (1982) and Fine and Clarkson (1982), who surmise that the observed patterns of infection are due to intensive mixing in secondary schools. It may well be the case that the aggregation and disassembly of schoolchildren is the underlying factor which precipitates the contact of infected with susceptible individuals and thus leads to the observed patterns of transmission of the influenza virus in a given population. However, if this was the case, the average age of infection could be expected to be lower than was found (between 20 and 30). This indicates that either the school system is not as responsible for the outbreaks as is suspected, or that the estimation of the average age made here is too high (probably due to over-reporting of cases in the elderly). It is therefore a priority to determine the average age of infection, as well as the age-dependent forces of infection, from serological data.

A recognised aspect of infection with the influenza virus is the acquisition of immunity to one strain of the virus after previous infection with an antigenically similar strain. Rigo-

rous studies on volunteer patients have been undertaken proving that rechallenge with homologous variants demonstrate a potent reinfection resistance that lasts for at least four years (Couch and Kasel (1983)). An indication that homotypic immunity to reinfection with the same subtype of type A virus may be prolonged (or even life long) was demonstrated by the reappearance of the H1N1 strain in 1977. Despite certain exposure to persons infected with the H1N1 subtype, individuals born before 1952 have rarely been infected with circulating closely related H1N1 virus strains. In the 1977/78 season 23% of H3N2 infection occurred in the 35+ age group, but only 2% of H1N1 occurred in the same age group of patients admitted to health care facilities in Houston, Texas (Glezen, Couch, and Six (1982)). Thus it seems clear that a substantial degree of immunity to reinfection with strains of the H1N1 subtype can persist for more than 20 years. This makes it practical to determine the infection history of an individual by analysing the antibody content of the individual's sera by means of an enzyme immunoassay.

From the analysis of serological data of this type it would appear that both the A/Hong Kong/68 and the A/Bel/1/81 strains were introduced into the population around 1968 and 1981, respectively. However, it appears that the A/Eng/333/80 strain generates a relatively high level of seroprevalence over a long time period, indicating that antibodies generated against a similar strain were circulating prior to 1980. Persistently high levels of seroprevalence suggest a maintenance through time of continuously high levels of viral transmission. The H1N1 sub-type, specifically, appears to be endemic for all the years considered in this study. This is also supported by the relatively high force of infection estimates found for children based both on case notifications and serological analysis. Thus it may be assumed that the population was exposed, in considerable magnitude, to the previous H1N1 strains, leading to raised antibody levels against all the H1N1 strains, and thus indicating that the A/Eng/333/80 (H1N1) was in fact approaching an endemic equilibrium. This apparent endemicity of the H1N1 strain after the initial occurrence is very surprising, considering that no significant record of illness during the off-season months has been recorded (Glezen,W.P. (1980)), and that after the initial epidemics, no significant case notifications occur (see chapter 3). One explanation is that the results observed reflect a lack of specificity in the serological test employed (described in chapter 4). However, this explanation

does not account for the epidemic appearance of the A/Bel/1/81 strain, nor does it seem likely in the light of the purification process that was employed to obtain the antigen for the serological test. Levels of antibody to the more recently introduced H3N2 strains (A/Hong Kong/68 and A/Bel/1/81), however, indicate that both these strains were epidemic, but tending towards an equilibrium of sorts, over the time period studied. The increased number of cases caused by the A/Eng/333/80 strain of the H1N1 sub-type was due almost entirely to infections in young persons, not previously exposed to this sub-type. This agrees with work done by Periera and Chakraverty (1982), who state that the A/Eng/333/80 epidemic consisted mostly of school-age individuals. There is evidence from the serological analyses that both the strains of H3N2 studied, during the years of their individual epidemics, caused significantly more infection in the older age groups than in the other age groups. This appears to confirm what was suspected during the analysis of case notification data; that these strains in particular cause an increase in the cases amongst the elderly, although caution must be exercised in the interpretation of case notification data (see section 7.2 for more detail).

Antibody titres remain high in all age groups for seropositive individuals, indicating that antibody directed against the influenza virus is long-lasting, and the concentration of individual antibody does not decline or vary excessively (see Fig 5.2). Therefore, it is possible that immunity to any one strain is life-long. However, there may be cell-mediated effects needed for complete immunity which become less effective through age. This could explain the higher level of incidence for all strains of the influenza virus observed in the elderly. It is interesting to note that the strain of H1N1 studied, A/Eng/333/80, which appears to be have been endemic for many years (at least 10), shows the lowest variability in antibody levels, and A/Bel/1/81, the most recent H3N2 strain, shows the greatest variability (see Fig 5.3). This further supports the hypothesis that the strains of virus appear to reach an endemic equilibrium in the community after the initial epidemic, although it must be noted that there is no evidence of persistent influenza cases during the off-season period, namely weeks 20-50 (PHLS, Chakraverty,P. et al. (1980), Glezen,W.P. (1984)).

From direct measurement of the proportion of the population which expresses antibody to a specific virus strain which is acting on a naive population it is possible to determine the age-dependant forces of infection for a particular strain in that season. This has been done

for the strains A/Hong Kong/68 and A/Bel/1/81; it is also possible to compare the pattern of the age specific forces of infection for A/Hong Kong/68 (Fig. 5.6(b)) with the patterns of infection found during the analysis of the case notification data (Fig. 3.6). Both patterns of infection show a peak around 20 years of age, followed by a high level of 'infectivity' in the 65+ age group. There is a different pattern observed in the case of the A/Bel/1/81 strain (Fig 5.6(b)), which shows a higher force of infection in younger individuals, followed by a gradual increase through age. Estimates for the A/Hong Kong/68 strain (Fig. 5.6(a)) agree well with the estimates from the endemic situation (Table 5.2). From the series of cross sections representing a 'longitudinal' study it was found that the age-dependent forces of infection are increasing with through time and hence with age continuously after the initial year of incidence for each strain. Thus, force of infection estimates obtained from the virus strains under consideration demonstrate age-dependency in transmission rates as well as variation through time after the year of initial incidence. The high estimate for the 15-20 year old age group can be contrasted with the lower estimates in the young, which suggests that the rates of mixing of individuals within age groups is far from homogeneous. An essential aspect of further studies of this sort must be the acquisition of sera sets with finer age-stratifications in the younger age groups. This would enable a more thorough analysis to be performed, with the potential to determine precisely the changes in the force of infection in a population through age (as determined from age-stratified serology).

The average ages of infection for the various strains (estimated from the proportion of each age group seropositive to each strain) are consistent with those recorded in other studies (see Table 3.1). From Fig 5.10 it can be seen that the average age of seroconversion remains relatively constant for both A/Eng/333/80 and A/Hong Kong/68, but is distinctly rising in the case of A/Bel/1/81. In all cases the average age of seroconversion lies between 20 and 30, which indicates that school-age mixing does not have as great an effect on the transmission as is generally believed (Fine and Clarkson (1982), Longini et al. (1984) and Glezen (1982)). These high values for the average age of infection also re-emphasise the effect of a virus being introduced into a population comprising mainly of susceptible individuals. Since the only immunity which acts against the 'new' strains of virus is that conferred by previous, antigenically similar strains, the proportion of older individuals who are immune

to the virus is less than that proportion observed for a virus which is at endemic equilibrium. In the latter case, the majority of older individuals will have been previously exposed to infection from the virus, causing the average age of infection to be considerably lower than is observed for the influenza virus.

Values for the basic reproductive rate were estimated from the serological data. It was shown in Table 5.2 that whereas A/Eng/333/80 (H1N1) and A/Bel/1/81 (H3N2) had R_{0i}' values of between 8 and 9, the A/Hong Kong/68 (H3N2) strain had a value of approximately 20. It is interesting to note that the A/Hong Kong/68 strain appears to have been circulating for longer than the other two by some 10 years. However, it is likely that the basic reproductive rate for most influenza A virus strains lies somewhere near 8 or 9. In terms of the overall reproductive rate of the influenza virus (R_0 ') it has been shown that, in the same way that increased infectivity of a virus in isolation acts to raise the reproductive rate, so do the infectivities of two strains of the same virus which are co-existing. Analysis suggests that cross-immunity between two co-existing strains causes the overall reproductive rate of influenza (R_0 ') to be elevated over that pertaining to any one strain. However, this elevation is in part a reflection of the method used to estimate R_0 ', based on the fraction susceptible to infection by either of two co-existing strains of the influenza virus. It seems that the fraction of the population susceptible at equilibrium is less when two strains are circulating than when any one strain is circulating in isolation.

The values recorded for the strain specific basic reproductive rates (approximately 10) are not exceptionally high by comparison with other viral infections, for example rubella. This virus has a R_0 value of approximately 5 and the average age at infection is approximately 9 years old (Anderson and May (1983)). Thus, although the basic reproductive rates are approximately the same, the average ages at infection are much higher for infection with the influenza virus. This may well be a consequence of the epidemic nature of the influenza virus; since it is generally introduced into a 'virgin' population all individuals should have an equal probability of becoming infected. This would then result in the average age at infection being decided almost entirely by the mixing patterns of the various age groups. Since the influenza virus, unlike rubella, does not persist in an endemic fashion in the population it is probable that all past experiences of infection with selected strains of the influenza virus

decrease the ability to invade supposedly susceptible populations. In other words, a new variant of influenza faces a not entirely susceptible population, but a host population with an existing partial immunity. As a consequence of partial immunity in some age groups, the average age of infection would rarely be uniform from one epidemic season to another.

In addition to affecting the average age of infection, the impact of partial herd immunity leads to considerable survival pressure being imposed on the influenza virus. As mentioned earlier, influenza has a genome composed of RNA rather than DNA. The primary stage in the replication of influenza is the synthesis of DNA from the RNA template provided by the viral genome, which is catalysed by the enzyme reverse transcriptase. Possible evolutionary relationships among retroviruses have been deduced from the differences among the sequences of amino acids that compose reverse transcriptase and also retrovirus proteases. These can be represented by 'phylogenetic trees' such as those shown in Fig 7.3. The phylogenetic tree for several years of evolution of the influenza virus is shown in Fig 7.3(a) (adapted from Both,G.W. et al. (1983)). If this tree is compared with a phylogenetic tree constructed for Human Immunodeficiency Virus Type 1 (HIV-1) isolates, which is the etiological agent for the Acquired Immune Deficiency Syndrome (AIDS) (Fig. 7.3(b)), an interesting feature is apparent. The phylogenetic tree of the influenza virus is far less 'bushy' than that of the HIV-1 virus, despite the fact that the data now available indicate that the two viruses exhibit roughly comparable rates of change in genetic composition, (of the order of 1 substitution per 100 nucleotides per year) (Myers,G.L. et al. (1989)). The changes in the genome of the influenza virus are manifest primarily as relatively infrequent (compared to HIV-1) appearances of different strains. This may suggest that the influenza virus is occupying an ecological niche in which competition among strains is intense. Conversely, the HIV-1 data suggests that, to date, there is not intense competition between HIV-1 strains. This in part must be due to the fact that infection with one strain does not confer protection against the emergence of an 'escape' mutant in an immunodeficient host (where the term 'escape' means an antigenically distinct strain). More explicitly the mutation events that trigger strain variation in HIV-1 all occur within the human host. For the influenza viruses this may not always be the case, since strains of influenza which are infectious to humans often originate in swine or avian hosts, and only become infectious to humans after several mutations.

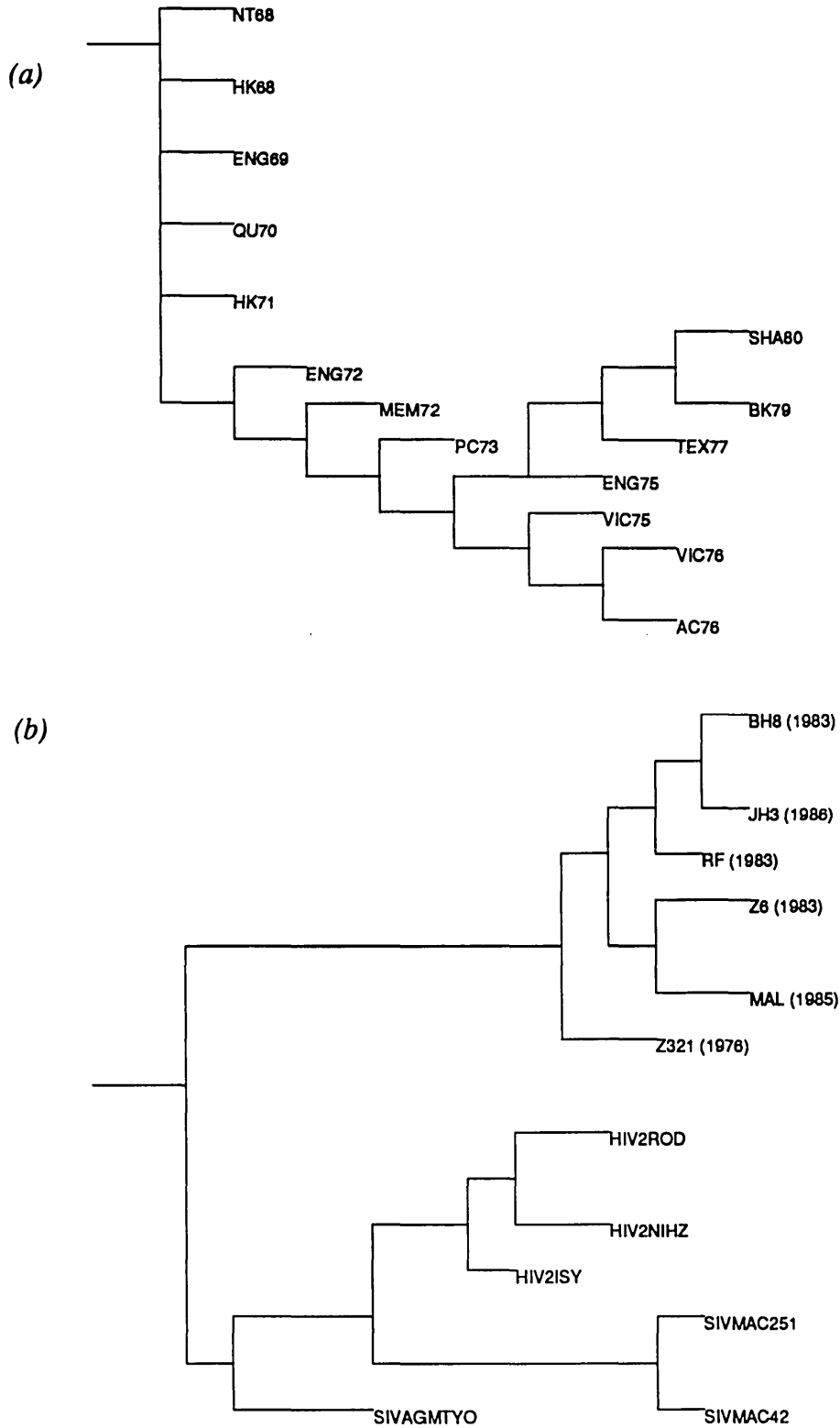*Figure 7.3. The comparison of two phylogenetic trees, (a) for the influenza A virus (after Both,G.W. (1983), and (b) for the HIV1 virus (from Myer,G.L. et al. (1989)). The horizontal distance between branches are approximately proportional to the genetic distance between the sequences of the nucleotides. Note that the tree for the influenza virus has less branching, and thus gives it a less 'bushy' appearance.*

7.2. Limitations of this study and areas for further research.

The most serious limitations to this work were imposed by the epidemic nature of the influenza A virus. As a consequence of this much of the mathematical techniques which have been developed to estimate useful epidemiological parameters such as the basic reproductive rate (R_0), and the average age of infection (A), could not be used. Therefore, the estimates which were made are very crude, and may not accurately represent the true situation.

The non-representative nature of the case notification records, and the age structure of the serological data base, was by no means ideal, being clumped into age groups which were too broad. To allow a detailed analysis of changes in the rate of infection by age it should be possible for the case notification data presented in both the PHLS reports, and in publications arising from these reports, to be obtained in a raw data format indicating the number of individuals who have suffered from the disease of influenza, with each individual's age being stated. This would enable more extensive analysis. However, because influenza is not a nationally notifiable disease, the problems of under- and over-reporting will always remain. Even if it was notifiable, problems remain concerning the precise diagnosis of infection without resort to viral isolation.

The analysis of case notification data is fraught with many problems in the case of influenza virus infection. The main problem with the case notifications is that influenza is not a nationally notifiable disease. This causes the resultant notifications to be variable, not only with respect to the seasonal magnitude of the epidemics, but also with respect to the number of cases recorded for each age group. The tendency will be for those age groups which are most at risk of developing complications (the two extremes of age; old and young, as discussed in detail in chapter 1) to be brought to the attention of a General Practitioner, and thus reported to the PHLS, more frequently than the age groups at a lesser risk. Due to the epidemic nature of influenza, the case notifications are not representative of the continuous acquisition of immunity to the virus through time, and therefore do not represent the gradual build up of immunity with age. As a consequence, it is not possible to build an 'age prevalence profile' for the various strains, which would be useful as a comparison to the serological work presented in chapter 5. It is thus only possible to make qualitative judge-

ments on the age-specific nature of the infectivity of these virus strains from the case notification data presented.

As mentioned earlier, the ideal serological data set consists of finely stratified age groups, covering all ages. However, it is increasingly obvious that the most important age groups to study are those consisting of individuals less than 30 years of age, which is the age span over which most of the change in the force of infection is observed. Therefore it would be useful to concentrate on these age groups during any future serological analyses. If this were done it would be possible to obtain both more precise estimates of the forces of infection in these age groups and a better understanding of the patterns of age-related change.

A problem encountered during the screening of the test samples was the potential cross-reactivity between the strains of the virus. In an attempt to eliminate this problem, the antigen was purified to the protein level, thus providing pure haemagglutinin for use in the serological test. Other serological studies of influenza have made use of the wide variety of techniques which give a good specificity in terms of subtype recognition, for example the Complement Fixation method (Julkunen, Pyhala and Hovi (1985), Julkunen, Kleemola and Hovi (1984)), the Haemagglutination Inhibition method (Sato, Ochiai and Niwayama (1988), Koskinen, Vuorinen and Meurman (1987)) and various Enzyme immunoassays (Van Voris et al. (1985)). However, although these tests return less error in terms of cross reactivity when used with whole virus, it is not practicable to screen the large number of sera samples that were screened in this study. As a consequence of these limitations, it was decided to use the ELISA technique in this study, with a highly purified antigen.

A significant difference was determined between the observed and expected values (calculated under the assumption of no cross-immunity between strains or cross-reactivity in the serological test) for all three strains tested for cross reactivity. In each case there were greater numbers of observed double-positive individuals than expected, which indicates evidence of cross reactivity. These values were recorded from the 1989 data set, and all other years showed a similar significant result. However, it must be remembered that any cross-reactive antibodies could, themselves, lead to a protective response. Whatever reason is behind the

cross reactivity, it is consistently stronger for A/Bel/1/81 interacting with A/Hong Kong/68 than with any other combination.

It would greatly simplify the analysis of serological data if laboratory tests could be designed which eliminated any potential cross-reactivity between similar antigens. Although tests of this sort already exist, such as the Haemagglutination Inhibition (HI) test described in chapter 4, these tests do not lend themselves to the screening of large sets of sera such as the one examined in this study. If an ELISA could be commercially produced which could distinguish between different strains of the same subtype of influenza A, the purification of the antigen would not need to be so thorough, and hence laborious.

Other problems with this type of screening include the definition of positive and negative values for the individuals under test, once the assay has been conducted on a sera set. Significant weight is added to the validity of the method used by the results of the analysis, which show a greater number of negative individuals in the younger age classes than in any other. This suggests that the antibody concentration cut-off points used are likely to be representative of the acquisition of infection. However, it cannot be ignored that some cross-reactivity between the serum samples occurred, considering the nature of the antigens being tested. Test sensitivity and specificity is impaired because the antibodies that lead to cross-immunity will, most likely, be cross-reactive to the various antigens. The problems involved with cross-immunity and the sensitivity and specificity of the test procedure cannot be resolved at present. Their resolution awaits a greater understanding of the mechanisms behind the immunity generated to different strains of the influenza virus. Until that time it will be necessary to analyse the results from studies of this type empirically and treat with caution any conclusions drawn regarding cross-reactivity between strains and the concept of cross-immunity. The estimation of a cross-immunity coefficient itself is also problematical, since it is not possible to determine exactly the amount of immunity which a particular strain confers on individuals exposed to other strains. However, an idea of the magnitude of this parameter can be estimated from case notification data simply by comparing the size of two epidemics (in terms of infected individuals) caused by different strains occurring in the same season.

It is an important area of further research to determine better estimation methods for R_0 and the average age at infection for viruses which are characterised by epidemic style patterns of infection (i.e. are introduced into a population consisting largely of susceptible individuals each season). At present most theory pertaining to the estimation of these parameters is based on the supposition that the virus is at an endemic equilibrium in the host population.

Much further work and analysis of the mathematical models, both in terms of equilibrium states and numerical studies, is needed. However the introduction presented in this thesis indicates that as it stands the model provides a crude understanding of the dynamics of co-existing virus strains (see Figs. 7.1 and 7.2). More importantly the model provides clues to the role played by cross-immunity. It would be useful, however, to be able to obtain more accurate estimates of the cross-immunity coefficient. Unfortunately the only way that this could be done is to compare the dynamical behaviour of one strain of a virus when it is in isolation to its behaviour when it co-exists with another strain. This would require studies in small local populations who have and have not been exposed to one, the other, or both strains. A final area for further study concerns the inclusion of seasonal effects, and more diverse mixing patterns into the numerical analysis of the two-strain age-structured model.

The simple mathematical model presented here can also be refined in other ways. The method used to included the effect of one strain conferring immunity to another, later strain, could be altered to include the reduction of the value for the transmission probability of the latter virus, and not simply set a fraction of the population to become immune to both strains of the virus after infection with the first. In this way cross immunity would also act to decrease β if a past infection with other strains does act to confer some immunity.

7.3. Conclusions.

The two main concepts under consideration here are; the effect of immunity conferred against one strain due to exposure of the host population to a previous antigenically similar strain of the same virus, and the result of a virus strain being introduced into an essentially immunologically naive population. Taken separately, these two situations provide interesting epidemiological scenarios, but in combination the results are highly complex in terms of the transmission dynamics of the virus, and hence difficult to interpret.

The serological studies suggest that the constant build up of antibody to co-existing strains of the same sub-type of the influenza virus (antigenic drift) eventually leads to a complete, generation-long, immunity to the subtype itself. This ensures that a new epidemic can only be generated by a new subtype as a result of significant mutation in the haemagglutinin gene (antigenic shift). The necessity for the emergence of a new antigenically distinct strain is probably the result of intense competition between strains of the same virus for a restricted ecological niche (susceptible hosts). The co-circulation of two (or more) strains causes a swift depletion of the susceptible individuals in a population. Thus, competition for resources (i.e. susceptible hosts) is increased by the presence of two co-existing strains that are antigenically similar, which leads to a combined infectiousness considerably in excess of a single strain in isolation. Therefore, in short, it is likely that the forces of infection, which characterise the transmission dynamics of a virus strain when it is circulating in isolation, are significantly modified when two closely related strains of the same virus are circulating. The net effect is for the two strains to more rapidly reduce the supply of susceptible hosts than would be the case for either strain circulating in isolation. In essence herd immunity to one viral strain acts to reduce the effective rate of transmission of a closely related strain, but the combined effect of the presence of both strains is to enhance the rate at which the supply of susceptible hosts is depleted. In such circumstances, the strain that dominates will be the one with the greatest reproductive potential. However, if cross-immunity occurs in the dynamical interaction with the host population, the strain that arrives first has an advantage over the strain that arrives later, making the establishment and persistence of the latter more difficult. The overall persistence of co-circulating stains from season to season will in

part depend on spatial factors (unsynchronised epidemics of the different strains in different localities). This aspect of influenza transmission requires further study by extending the mathematical models to include spatial dynamics. With respect to epidemiological study, spatial factors could be examined by studying case notification and serological trends in small communities. This would require the stratification of such data by town, region or area within the United Kingdom. This is a major priority for further research.

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Appendix A (Experimental Buffers).

Phosphate Buffered Saline (pH 7.6).

85g NaCl
12.8g Na_2HPO_4
1.56g $NaH_2PO_4.2H_2O$
10l Distilled H_2O

Coating Buffer (Carbonate Bicarbonate; pH 9.6, 0.05M).

1.59g Na_2CO_3
2.93g $NaHCO_3$ Used for antigen coating.
1l Distilled H_2O

Incubation Buffer.

900ml PBS   Used for serum and conjugate dilution.
0.45ml Tween 20   Blocking protein included in some assays.

Substrate Buffer (Phosphate Citrate).

7.19g Na_2HPO_4
5.19g Citric acid
1l Distilled H_2O

Washing Buffer.

90g NaCl
5ml Tween 20   Used for washing plates.
10l Distilled H_2O

Substrate Stock.

100mg orthophenylene diamine (OPD)
10ml methanol

Stored in dark at $4^{\circ}C$ for a maximum of 1 week.

Substrate Working Solution.

100ml Substrate Buffer
1ml OPD Stock
50ul 6% H_2O_2

Made up immediately before use.

* All buffers were made up freshly before each run and renewed fortnightly.

Appendix B (Abreviations used in the text).

APP = Age-Prevalence Profile.
ASP = Age-Serological Profile.
BSA = Bovine Serum Albumin.
CDSC = Communicable Disease Surveillance Centre.
EDTA = Ethylenediamine tetra-acetic acid.
ELISA = Enzyme-Linked Immunosorbent Assay.
FOI = Force of Infection.
HA = Haemagglutinin.
HU = Haemagglutinating unit.
IgA = Immunoglobulin type A.
IgG = Immunoglobulin type G.
IgM = Immunoglobulin type M.
mg. = milligram.
ml. = millilitre.
NA = Neuraminidase.
nm. = nanometer.
OPD = Orthophenylene diamine.
PBS = Phosphate Buffered Saline.
PHLS = Public Health Laboratories Service.
RBC = Red Blood Cell.
SDG = Sucrose Density Gradient.
Tris = Tris(hydroxymethyl)methyline.
ug. = microgram.

Appendix C (Mixing Matrices).

Mixing Patterns for the 'Who Acquires Infection from Whom' (WAIFW)[*] matrices used in the age-structured compartmental deterministic model.(From Anderson and May (1985)). The matrices represent the degree of mixing between susceptible and infectious individuals of different age classes within a population. They are used used to determine the age-specific forces of infection used in the mathematical model described in chapter 6. The age classes 1 to 5 thus represent the ages 1-, 5-, 10-, 15- and 55-75 respectively.

WAIFW A

INFECTIOUS INDIVIDUALS

Age Class	1	2	3	4	5	
1	$\beta 1$	$\beta 2$	$\beta 3$	$\beta 4$	$\beta 5$	S
2	$\beta 2$	$\beta 2$	$\beta 3$	$\beta 4$	$\beta 5$	U S C E P T I B L E S
3	$\beta 3$	$\beta 3$	$\beta 3$	$\beta 4$	$\beta 5$	
4	$\beta 4$	$\beta 4$	$\beta 4$	$\beta 4$	$\beta 5$	
5	$\beta 5$	$\beta 5$	$\beta 5$	$\beta 5$	$\beta 5$	

WAIFW A

INFECTIOUS INDIVIDUALS

Age Class	1	2	3	4	5	
1	$\beta 1$	$\beta 1$	$\beta 3$	$\beta 4$	$\beta 5$	S U S C E P T I B L E S
2	$\beta 1$	$\beta 2$	$\beta 3$	$\beta 4$	$\beta 5$	
3	$\beta 3$	$\beta 3$	$\beta 3$	$\beta 4$	$\beta 5$	
4	$\beta 4$	$\beta 4$	$\beta 4$	$\beta 4$	$\beta 5$	
5	$\beta 5$	$\beta 5$	$\beta 5$	$\beta 5$	$\beta 5$	

WAIFW A

INFECTIOUS INDIVIDUALS

Age Class	1	2	3	4	5	
1	$\beta 1$	$\beta 1$	$\beta 1$	$\beta 4$	$\beta 5$	S U S C E P T I B L E S
2	$\beta 1$	$\beta 2$	$\beta 3$	$\beta 4$	$\beta 5$	
3	$\beta 1$	$\beta 3$	$\beta 3$	$\beta 4$	$\beta 5$	
4	$\beta 4$	$\beta 4$	$\beta 4$	$\beta 4$	$\beta 5$	
5	$\beta 5$	$\beta 5$	$\beta 5$	$\beta 5$	$\beta 5$	